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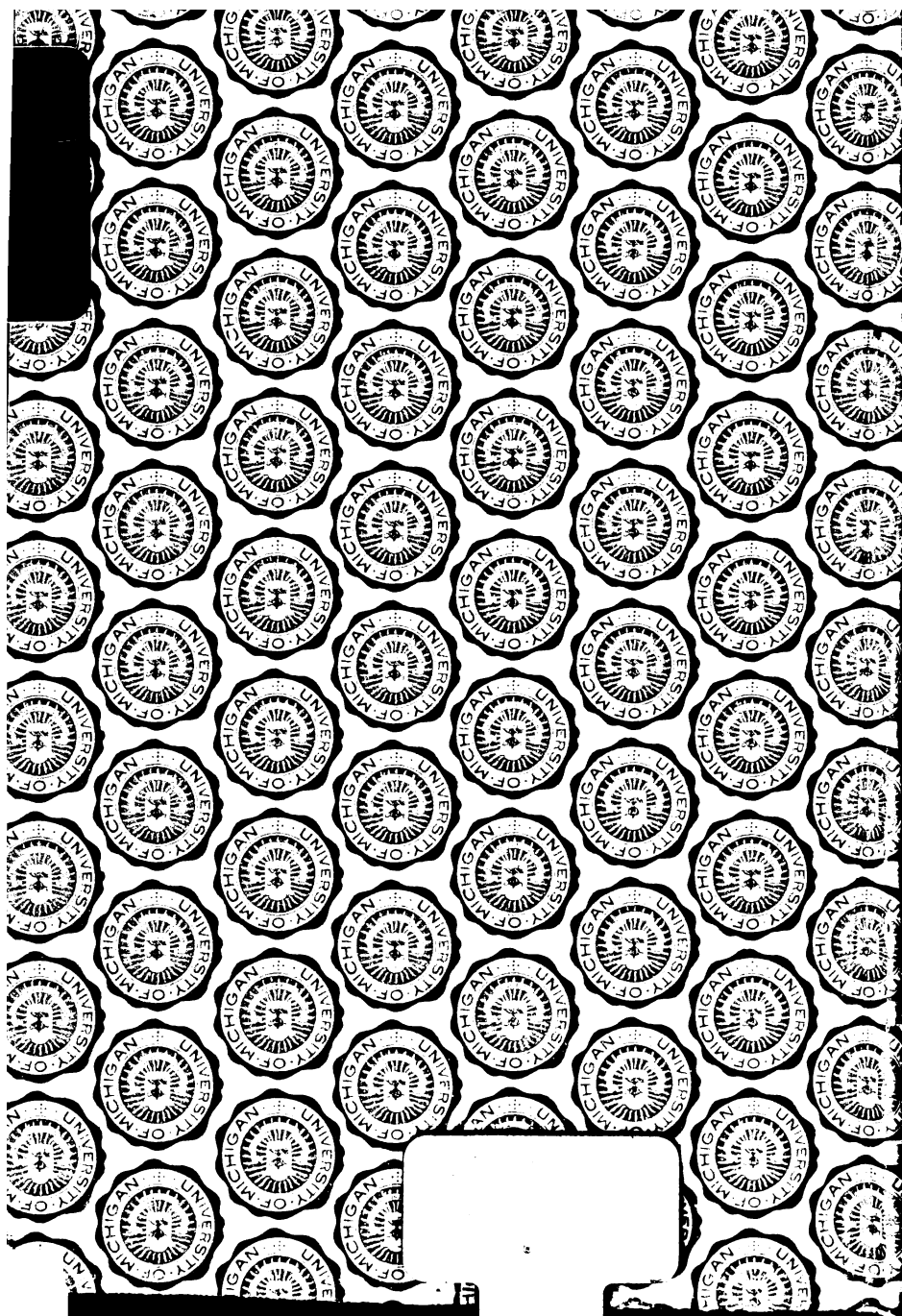
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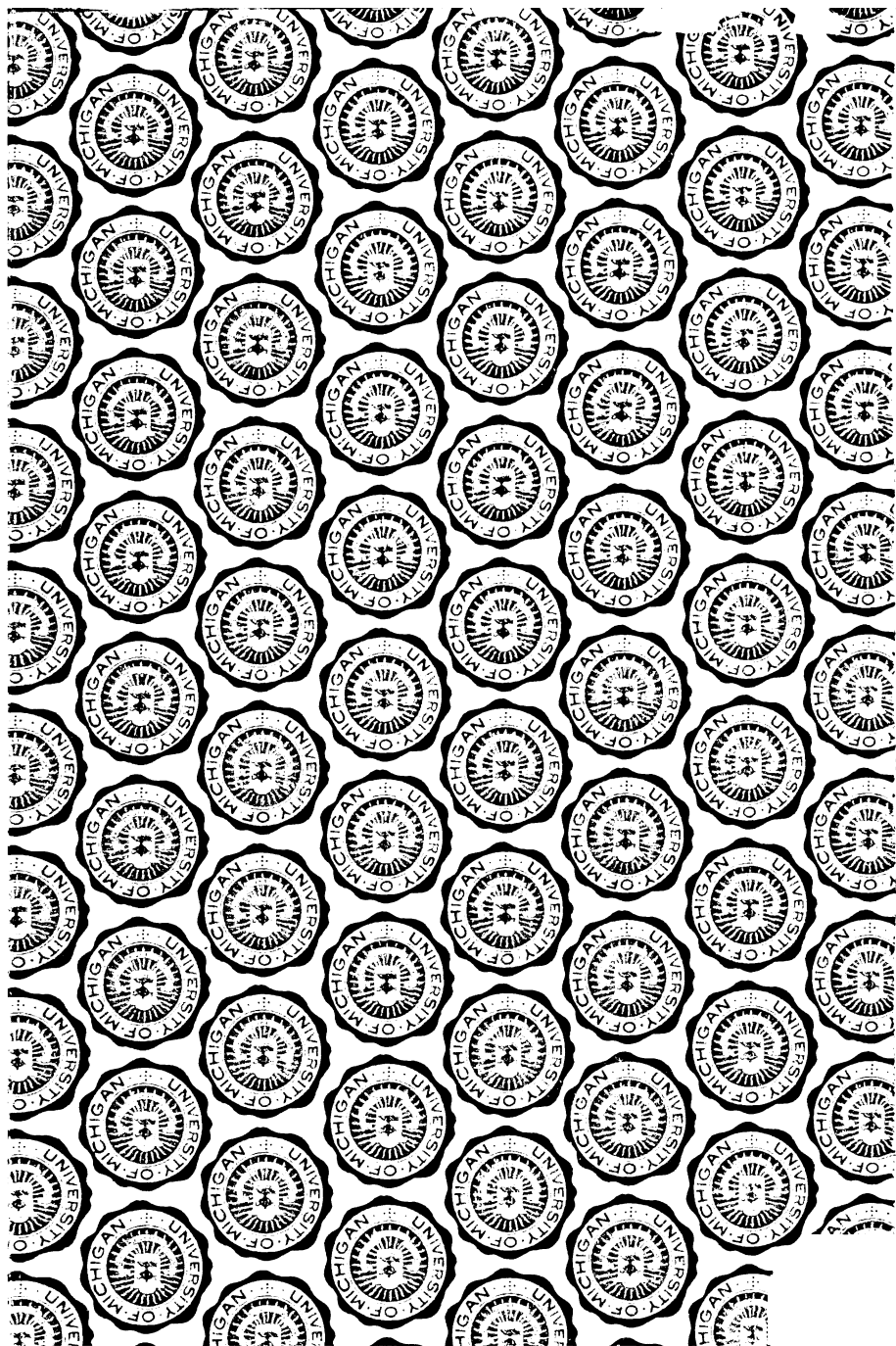
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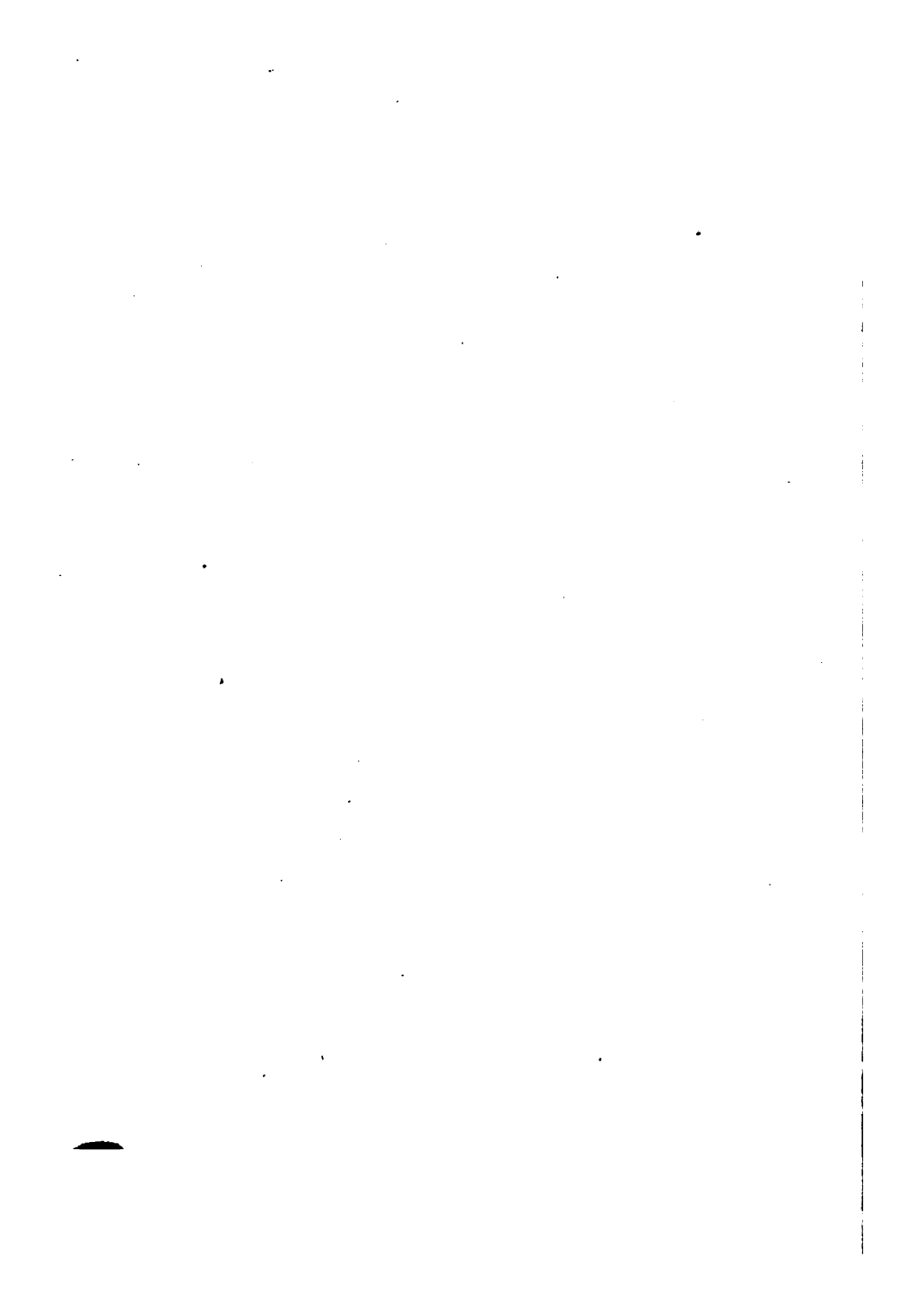
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**PRACTICAL**  
**STANDARDIZATION BY CHEMICAL ASSAY**  
**OF**  
**ORGANIC DRUGS AND**  
**GALENICALS**

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**A MANUAL FOR THE STUDENT OF PHARMACY AND A CONVEN-  
IENT HAND-BOOK OF PHARMACEUTICAL ASSAYING AND  
STANDARDIZATION FOR THE PRACTICAL PHARMA-  
CIST, THE MANUFACTURER, THE CONTROL  
CHEMIST OR THE DRUG INSPECTOR.**

**BY**  
**A. B. LYONS, F. C. S.**

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**DETROIT**  
**NELSON, BAKER & CO.**  
**MANUFACTURING PHARMACISTS**

**1920**

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## FOREWORD

By standardization of a drug or medicinal preparation we mean bringing it to a definite strength as regards its medicinal activity. There are two ways in which this may be attempted. We may either make a direct trial of the effect produced by the medicine, administered under certain specified conditions, or we may ascertain if possible what is the active constituent of the drug, and then determine by assay the proportion of this constituent present in the sample under examination. The former method is empirical of course, but not on that account necessarily unscientific. The practical difficulty in endeavoring to apply it is that we have to deal with the reactions of a living organism to influences the nature of which is at best imperfectly understood. It is evident that we cannot hope to secure anything like identity in the conditions under which our experiments must be made, and the assumption that the reactions observed in a subject in normal health are not radically different from those under which drugs produce their therapeutic effects in illness is to say the least, a questionable one. Further it is obviously impracticable to employ the human subject in experiments of this character and the inferences deduced from experiments on the lower animals may easily be exceedingly misleading. Hence it is only to a very limited extent that the so-called biological method of assay is practised. The U. S. Pharmacopœia gives it recognition, it is true, but in only one instance does it make a biological assay mandatory, and even in that instance offers a standard so vague that experts disagree widely in the results they report.

Standardization of any drug implies the possibility of a reasonably exact determination of its active constituent or constituents. Trustworthy assay processes therefore are the one solid foundation on which

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to build. That the results of such assay shall be minutely exact is not essential, but the results must not be of doubtful interpretation. It sometimes happens that no assay has been found which is in itself infallible, but that we have two processes mutually confirmative, by which practical certainty of our results may be reached.

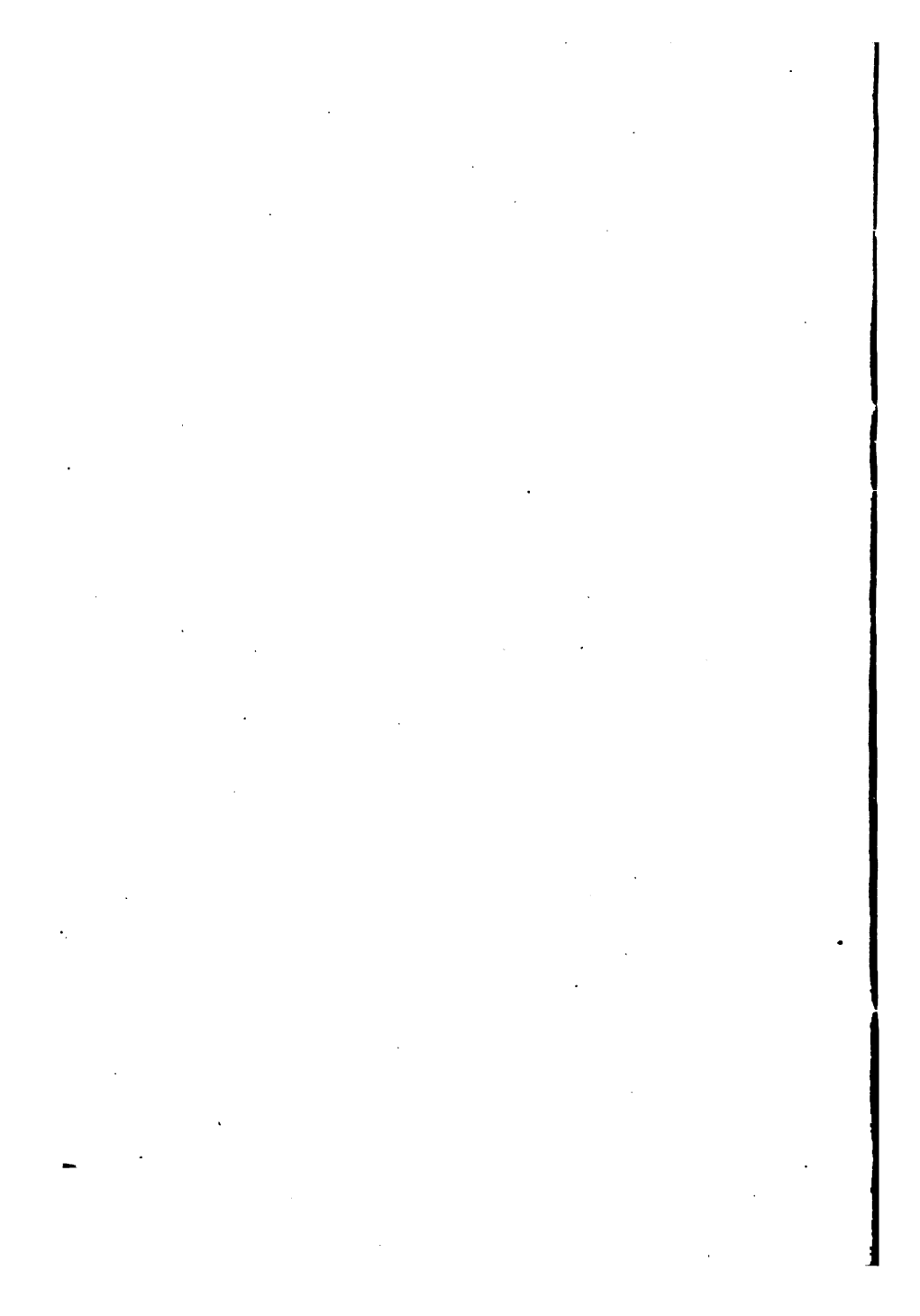
In preparing this brochure reasonable completeness has been the aim kept in view, but in this age of rapid progress in science and in the practical application of scientific discoveries, completeness is an ideal that may be approximated perhaps but never fully attained.

Grateful acknowledgement is due to a number of friends who have rendered valuable aid in the task undertaken. The alcoves of the University library at Ann Arbor have been made accessible through the courtesy of Dean A. B. Stevens of the Michigan University School of Pharmacy. Many helpful suggestions have come from my old colleague on the U. S. P. Revision Committee, Wilbur L. Scoville, who has also done faithful and efficient work in revising the proofs. Dr. W. S. Hubbard likewise has rendered similar assistance, and is further to be credited with having been the first to suggest the undertaking by one nearing his four score years of so arduous a task.

Finally, I realize that such a work must depend for its value on experience gathered during quarter of a century in the laboratory of Nelson, Baker & Co. and on the importance attached by that house to standardization of its products.

Laboratory of Nelson, Baker & Co., June, 1920.

**Part I.**  
**General Principles and**  
**Procedures**





## PRELIMINARY OPERATIONS IN ASSAY OF CRUDE DRUGS

1. **For crude drugs** pharmacopoeial requirements as a rule are content with prescribing in lieu of a standard, a minimum content of active principle. Exceptionally the drug in powdered form is brought to a definite absolute standard—as in the case of Opium. The value of the drug, however, in any case must be determined by assay. If the result of such assay shows that the drug is below standard, it cannot be used in official formulas, but it still may be employed in other ways, e. g., as a source of alkaloids or other active principles.

2. **Selection and preparation of the sample.** It is vitally important in the first place to secure a fair average sample of the drug. When this consists of seeds, leaves, etc., nearly uniform in character, this is a simple matter. From different parts of each package a handful is taken, making sure that the interior and the superficial portions are equally represented, the whole is mixed and a suitable quantity, generally about fifty grammes, is taken as the sample for assay. By aid of mill, mortar and sieve, the whole of this portion is reduced to a powder of the requisite fineness.

3. In general it may be said that the finer the powder the better. The coarsest powder admissible is a No. 30 (in case of leaves of loose texture). For barks, seeds, etc. having a compact structure a much finer powder—No. 60 to No. 80—is generally required.

4. **If the drug consists of large pieces**, variable in quality, like rhubarb and most barks and tubers, it is best to select first a sufficient number of representative pieces. Take from each of these a representative section or segment, reduce all to a coarse powder, mix well and of this take about 50 grammes,

the whole of which is to be reduced to a fine powder, to provide the sample for assay.

5. **If the drug requires to be dried** before grinding, the loss of weight during this operation must be noted, and the requisite correction made in the result of the assay. Vegetable crude drugs generally are more or less hygroscopic, gaining weight in a damp atmosphere and losing in dry weather. The object of an assay is usually to ascertain the proportion of active principle present in the drug **in the condition in which it is purchased or used.** Here at the outset is introduced into the assay an element of uncertainty which is intolerable to the professional chemist. If the weight of the material assayed is subject to variation amounting possibly to five per cent, it is clear that the result of the work will be an approximate, not an exact figure. The ideal of the chemist requires for a basis the thoroughly dried drug as a positive invariable quantity. In practice we deal with the variable, hygroscopic drug, and must be content with practical, not ideal results, exactly as the practical mechanic uses for the fine measurements by which he adjusts part to part in a piece of delicate machinery, instruments of "precision" indeed, but confessedly only of practical and approximate, not of scientific and absolute, precision.

6. Of course in the case of a drug not to be used at once, particularly if it is to go into the market with a guaranteed assay, the moisture in the drug must be determined, and the completely dried drug must be made the basis of the percent of active principles reported.

7. **Methods of exhausting the drug.** The choice will be between four processes, each of which may have in particular instances its superior advantages.

8. **A. Maceration.** The drug in fine powder is placed in a bottle or flask, with a measured or weighed quantity of the chosen menstruum sufficient to ensure complete exhaustion—at least ten times the weight of the drug—the flask is securely corked and the drug is allowed to macerate therein with frequent or contin-

uous agitation several hours at least—in many cases from one to three days. A mechanical shaker reduces materially the time required for this operation, which commends itself by its simplicity, and consumes a minimum amount of the operator's time and attention.

**9. B. Percolation.** Details of this familiar pharmaceutical operation are hardly necessary here. Exhaustion of the drug can generally be effected in a shorter time by this process than by maceration. The strong percolate which passes at first should be reserved, and only the weaker percolates exposed to possible loss or change of their active constituents by heat applied to effect concentration. The method is the one most commonly chosen when the menstruum is aqueous, alcoholic or hydro-alcoholic.

**10. C. Boiling** with several successive portions of the chosen solvent. This is efficient and rapid, but generally more troublesome than the following method. Neither of these two methods are applicable where heat injures or destroys the active principle of the drug.

**11. D. Continuous Extraction by Repercolation.** This is altogether the neatest and most effective mode of applying solvents. It requires a special extraction apparatus ("Soxhlet"), and it takes a little time to mount this properly with condenser, but it enables us in a minimum of time and with a minimum of solvent, to exhaust the drug. When once set in operation, the action is automatic, so that the method is after all economical of time, particularly where assays require to be made frequently.

**12. The choice of solvent** will depend of course on the nature of the drug. In most cases the active principle is an alkaloid, which is present in the form of a salt soluble in water or alcohol. If we employ water as the solvent, we extract much inert matter, which is sure to embarrass subsequent operations. Alcohol is to be preferred, of at least 50 per cent strength—the stronger the better as a rule, unless the drug contains much inert resinous matter. Strong alcohol, however, may fail to penetrate the drug unless it is first moistened with water.

13. A mixture of alcohol and chloroform generally extracts alkaloids and glucosides from crude drugs very completely and leaves behind most of the inert matter, if this is not resinous in its nature. The addition of a little water of ammonia (or spirit ammonia) may be advantageous. Acidulated water or acidulated alcohol was formerly much used in exhausting alkaloidal drugs. A freely soluble salt of the alkaloid is formed, and the drug is speedily and very completely exhausted, but much inert matter is removed at the same time, and the plan is now seldom used.

14. Another plan is to treat the drug (alkaloidal) first with an alkali, milk of lime or of magnesia, or a solution of sodium carbonate, thus setting free the alkaloid. The dried mixture is then exhausted with strong alcohol or wood spirit, or other suitable solvent. The method is not to be recommended unless possibly for Cinchona bark. The objections to it are; 1st, the liability to decomposition of sensitive alkaloids by the prolonged action of the alkali, particularly when lime is used; 2nd, the amount of time required to carry out the successive steps of the assay.

15. For alkaloidal drugs the solvents now almost universally employed are modifications of the mixture recommended some years ago by Prollius for the assay of cinchona bark, known as the **Prollius mixture**, which was made in accordance with the following formula:

Concentrated Ether.....	325 mls
Alcohol.....	25 mls
Stronger water of Ammonia.....	10 mls

16. The Ammonia sets free the alkaloids in the tissues of the drug and these are then very rapidly taken into solution by the ether. It is to be understood that this solvent is used by the method of maceration. It is surprising how quickly and completely it effects the extraction of the alkaloid, particularly when aided by the mechanical shaker.

17. It was the present writer who first recognized the general applicability of the Prollius method to the assay of alkaloidal drugs, modifying the formula b4

replacing the other as occasion might require with some other immiscible solvent—usually a mixture of ether with chloroform.

18. **Keller later modified the procedure** by using a measured volume of the immiscible solvent—generally ether or ether-chloroform—then, after allowing a few minutes for penetration of the tissues of the drug with the solvent, adding a certain amount of water of ammonia, which was supposed to be absorbed by the drug without affecting the volume of the solvent. After the maceration, water was added to the mixture to be taken up by the drug, causing it to aggregate and so permitting the immiscible solvent to be drained off quite clear. Of course, when the solvent is ether, there will be a partial solution of each fluid in the other, so that the volume of the ethereal solution will not be exactly what it was when measured and it may be expected that on this account, as well as because of the great volatility of ether, the result of the assay will be high, if an aliquot portion of the ethereal solution is taken for the determination, as the author directs. The error, which is possibly more than compensated by the incompleteness of the extraction of alkaloid from the drug, is generally regarded as negligible in practice. However, this source of error may be eliminated by following the suggestion of Puckner, to drain off the whole of the ethereal fluid, transfer the drugs to a suitable percolator (a glass funnel whose neck is plugged moderately firmly with absorbent cotton answers the purpose fairly well) and extract the remaining alkaloid by percolation with the immiscible solvent. In the alkaloidal assay processes of the U. S. Pharmacopoeia, an aliquot portion (by volume) of the ethereal solution is taken, and this is in accordance with general usage.

19. We have now a solution which contains the alkaloids of the drug together with chlorophyll, fatty and waxy matters, volatile oils and a variety of other substances from which the alkaloids must be separated.

20. **Routine Process for extracting** from the primary immiscible solvent in relatively pure form the alkaloids present.

**21. Case 1. The immiscible solvent is specifically heavier** than water, so that when separated it occupies the lower part of the separator. We may assume that we start with the "ethereal solution" of (18), measuring 50 to 100 mils and representing 10 grammes of crude drug. The alkaloid in such a solution is present in the free state, but is accompanied by chlorophyll, fixed and volatile oils, resinous and waxy matters and other like impurities. To separate it from these, advantage is taken of the fact that acidulated water dissolves nearly all alkaloids freely leaving the impurities in the immiscible solvent. Repeated shaking out therefore with acidulated water, extracts the whole of the alkaloid, which can then be recovered in relatively pure condition by adding an alkali and shaking out once more with the immiscible solvent.

**22. Provide four separators,** (separatory funnels) into the first of which (No. 1) is introduced the ethereal solution. Add 15 mils of a 2 percent solution of sulphuric acid and shake gently one full minute. If there is any tendency to the formation of an emulsion, use a gentle rotary motion, continuing this for two minutes.

**23.** Set the separator aside until the fluids have separated completely, then draw off the lower layer into separator No. 2, add to this 8 mils of the 2 percent sulphuric acid and shake out as before, transferring the lower layer after separation to No. 3. Shake this out exactly as before with 8 mils of the acid, drawing off the separated lower layer into No. 4. The alkaloid has probably been practically all removed by this time, but shake out once more with 5 mils of the acid, and test 0.5 mil of the acid solution after separation with Mayer's or Wagner's reagent. If more than a faint cloud is produced, extraction of the ethereal solution must be carried to completion, but this will only exceptionally be necessary. Meanwhile the acid solutions in separators 1, 2, 3 and 4 have been washed in succession with 10 mils of chloroform (or of the original immiscible solvent) to remove impurities, the chloroform being finally rejected.

**24. Sulphuric rather than hydrochloric acid** is chosen for extracting the alkaloids because the alkali sulphate formed is not taken up to an appreciable extent by the solvent used for the final extraction of the alkaloid. Only in a few cases is the sulphuric acid inadvisable on account of the sparing solubility of the alkaloid sulphate. In such case another acid must be selected, but the final solution of the purified alkaloid must be washed (with ammoniated water) with unusual care.

**25. We have our alkaloids now in acid solution** in the several separators, No. 4 probably containing no more than a trace. Now add to separator No. 4, 25 mls of the appropriate immiscible solvent and 5 mls of water of ammonia, shake slightly and immediately transfer the entire mixture to No. 3, again shake and transfer to No. 2; introduce into the separator a bit of litmus paper; if this shows acidity, add more ammonia, making the mixture strongly alkaline, shake well and transfer the mixture to No. 1. Add more ammonia if necessary to insure alkalinity and shake carefully at least one minute. Draw off the ethereal layer containing most of the alkaloid into separator No. 4, following this with about one mil of the immiscible solvent to rinse the tube of the separator, add to No. 4, 10 mls of water and shake slightly, to wash out water-soluble impurities. When the fluids have separated, draw off the lower layer through a pledget of absorbent cotton into a tared beaker, following again with about 1 mil of the immiscible solvent.

**26.** Meanwhile add to separator No. 3, 20 mls of the immiscible solvent, transfer this to No. 2, then to No. 1, in which it is to be shaken well with the fluid therein contained, and after separation drawn off into No. 4, to be washed as in the former instance, being finally drawn off through the absorbent cotton into the beaker. It is not necessary to rinse the tube of the separators as in the first instance, since the quantity of alkaloid present is comparatively small. A third and fourth portion of the immiscible solvent (20 and 15 mls respectively) added directly to No. 1, will generally suffice to complete the extraction of

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the alkaloid, but make sure of this by testing 2 mls of the last portion for alkaloid by evaporating to dryness at a gentle heat, taking up the residue with a couple of drops of 5 per cent sulphuric acid, and a few drops of water, and adding Mayer's solution or other appropriate reagent. Finally evaporate the solution to dryness; if the solvent consists wholly or partly of chloroform, take up the residue with about 2 mls of neutral alcohol and dry again, repeating this once or twice in case the alkaloid is one (like berberine) which forms a combination with chloroform. Dry the residue at 100° C.\* to constant weight and weigh the residue as crude alkaloid. It is still not free from impurities and is reserved for a more rigid determination of the quantity of pure alkaloid it contains.

**27. Case 2. The immiscible solvent is lighter** than water, so that when separated it forms the upper stratum of fluid. Provide three separators, into the first of which (No. 1) is introduced the alkaloidal solution. Add 15 mls of 2 percent sulphuric acid, and shake gently one minute as in Case 1. After complete separation of the fluids draw off the lower aqueous stratum into separator No. 2. Should partial emulsification have taken place, draw off the emulsified portion with the clear fluid. Follow with about 1 mil of distilled water, to rinse the tube of the separator. Add to the second separator 15 mls of ether and shake well (not too vigorously). When separation has taken place, draw off the clear part of the lower stratum into No. 3, following with about 1 mil of water to rinse the tube of the separator. Add to separator No. 1, 5 mls of the 2 per cent sulphuric acid; shake out as before, transferring the separated fluid successively to No. 2 and No. 3, but not rinsing the tubes of the separators unless the quantity of alkaloid to be extracted is large. Repeat the extraction in precisely the same manner with successive portions of acid as long as alkaloid is taken up. Test this by adding to a few drops of the acid fluid of

\*A few alkaloids, like cocaine, will not bear so high a temperature. For volatile alkaloids a special procedure is required.



the fourth extraction a drop or two of Mayer's reagent or of Wagner's reagent (the latter imperative when caffeine is the alkaloid present).

28. **Finally extract the alkaloid** in separator No. 3, by shaking out with successive portions (25, 20, 20 and 15 mls—more if needed) of the immiscible solvent, after adding excess of water of ammonia. It is convenient to use for this two separators. The aqueous layer after each extraction is transferred from one separator to the other, while the upper layer is drawn off through absorbent cotton into a tared beaker. A drop or two of the aqueous fluid may possibly be carried over into the beaker but it is easy to decant the ethereal fluid into a second tared beaker in which it is to be evaporated. In such case rinse the first beaker with the immiscible solvent to be used for the next extraction, so that no loss of alkaloid is permitted.

29. **Whenever an alkali carbonate or bicarbonate** is used to set free the alkaloid, whether under Case 1 or Case 2, it is advisable to wash each portion of the final alkaloidal solution with 10 mls of water and then pass it through a dry filter before evaporating. Avoid allowing any visible drops of the aqueous fluid to go into the filter, which must finally be washed with ether or the appropriate immiscible solvent, the washings of course to be added to the alkaloidal solution.

30. **Emulsification in shaking out operations** is of frequent occurrence and causes the analyst much annoyance and loss of time as well as of temper. Certain drugs are particularly troublesome in this respect. The experienced operator will be constantly watchful for indications of any tendency in this direction and will frequently be able to overcome the tendency. This can often be done by increasing largely the quantity of the volatile solvent, ether and alcohol having particularly a favorable effect. If complete emulsification takes place, as it sometimes does quite suddenly, it is best to start the assay over again. Otherwise one may try the effect of heat, or of the addition of a few drops of alcohol. An obsti-

nate emulsion may sometimes be separated by the centrifuge, or by filtering with suction through a plug of absorbent cotton. When the emulsion is alkaline, as is most frequently the case, the addition of a small excess of acid will often cause separation. If the expedient is successful, add a large volume of ether and then proceed cautiously to render it again alkaline, but rotate rather than shake the separator. It may be necessary to evaporate the mixture, render the solution acid and treat it with lead acetate or subacetate, but no general rules can be laid down. See (115). *Tartrate Acid*

31. **Partial emulsification** is of very frequent occurrence. Sometimes it may be overcome by introducing into the mixture a pledget of absorbent cotton and stirring it about with a wire or a glass rod. The most successful procedure in the case of an alkaline mixture is to draw off the emulsion with the ethereal fluid (or draw off the clear portion of the aqueous solution), shake the emulsified portion with the ethereal fluid, when it will usually be at least partially separated, separate the ethereal fluid and add to the emulsion a fresh portion of immiscible solvent, which is to be shaken with the emulsion and after separation to be used in extracting the clear portion of the aqueous fluid. As a rule a new emulsion will not form, and we may proceed in a similar manner with successive additional portions of the solvent:

32. In the case of an emulsion **in an acid solution** add to the emulsified portion a few mls of 5 percent sulphuric acid, and evaporate off the volatile solvent by a gentle heat, stirring the mixture with a glass rod. Filter the acid solution, redissolve the residue in the ethereal solvent and treat the solution with acidulated water. Repeat this as long as alkaloid is taken into solution by the acid.

33. **It happens not infrequently** that when removal of the alkaloid from an alkalized solution is practically complete, an obstinate emulsion suddenly forms. In such a case draw off the mixture into a beaker and stir into it a gram or more of pow-

dered gum tragacanth. A thick mucilage is formed, from which by stirring with a glass rod, the immiscible solvent separates as a perfectly clear fluid. Obviously the expedient cannot be resorted to as long as the aqueous solution contains a notable quantity of alkaloid.

**34. Alternative Procedure.** Instead of shaking out the alkaloid from the original solution in an immiscible solvent, it is sometimes advantageous to proceed as follows: Evaporate off the solvent by exposure to a very gentle heat in a shallow capsule or evaporating dish. When the alkaloid is one particularly unstable, it is best merely to expose the fluid to a current of warm air. When the solvent has evaporated, add 5 mls of 5 per cent sulphuric acid and 10 mls of ether. Stir to redissolve in the ether the oily and waxy matter which may have separated, and to insure combination of all alkaloid with acid, evaporate the ether by aid of a current of warm air, then filter. Treat the residue in the capsule with 3 mls of slightly acidulated water and 5 to 10 mls of ether. Evaporate off the ether and filter the solution through the same filter as before. Repeat this operation once more, and note whether the solution obtained shows reaction for alkaloid. If more than a trace is shown, the operation should be repeated, but this would rarely happen. In any case, pass the solution through the filter. The alkaloid is to be extracted from the combined solutions in the usual manner.

The procedure is advantageous in case of some drugs containing very small quantities of alkaloid and in some which are troublesome about the formation of emulsions.

**35. The use of a salt solution** to facilitate extraction of alkaloids by the shaking out process (51) is highly commended by C. A. LaWall, whose procedure is as follows: Dissolve 25 gm. of sodium chloride in a 100 mil graduated stoppered cylinder, in water sufficient to make 85 mls, add 10 mls of the fluid extract to be assayed and make up the volume

with water to 100 mls. Agitate well for about one minute. Let stand five minutes, agitate again and pour on a dry filter. Collect 50 mls of the filtrate, representing 5 mls of fluidextract, and shake out in the usual manner with the appropriate immiscible solvent. In the case of fluidextract of guarana, use only 5 mls of the fluid and acidulate the solution slightly. The method does not succeed well with fluidextracts of cinchona, but is otherwise very generally applicable. It is convenient to keep on hand a solution of sodium chloride, prepared by dissolving 300 gm. of the salt in water sufficient to make a liter.

## THEORY OF SHAKING OUT PROCESS

36. **When an aqueous solution** of any substance is shaken with an equal volume of an immiscible solvent, the latter takes up the substance in solution until a condition of equilibrium is reached, each fluid containing a certain definite proportion of the substance, whatever the actual quantity of the dissolved substance may be (provided the temperature is the same). In the case of benzoic acid, ether and water, that proportion is 80:1. The **coefficient of saturation** is therefore  $1 \div 80 = 0.0125$ . A solution measuring 50 mls and containing 81 mg. of the acid would yield to an equal volume of ether at standard temperature 80 mg. and no more, if the shaking is carried to complete saturation. A second shaking out with fresh ether would result in the extraction of 80 parts out of 81 of the residual acid, i. e. of 0.9876 mg. of the acid, leaving behind only 0.0124 mg.\*

37. **If the volume of ether is one-fifth** that of the aqueous solution, the same principle will hold with regard to the distribution of the benzoic acid between ether and water, so that out of a total of 85 parts of acid, the water will retain 5 parts or one seventeenth, the ratio standing 16:1. It is evident that if the coefficient of distribution is known in any given case, a single shaking out operation with equal volumes of solution and immiscible solvent, provided the substance be in the outset wholly in solution and the shaking be continued until equilibrium is established, will by a simple calculation, give the total quantity

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\*It is to be understood that the law here stated applies only when the substance to be shaken out is actually in solution to begin with. If an alkaloid separates as a precipitate when the solution containing it is rendered alkaline, this precipitate must be wholly dissolved by the immiscible solvent before the law of distribution between solvents in a constant ratio comes into play. There is accordingly a distinct advantage in diluting the original solution sufficiently to prevent separation of a precipitate, unless such precipitate is quite freely soluble in the immiscible solvent.

of dissolved substance. For example, if the coefficient of saturation is 0.021, and the shaking out has extracted 116 mg. the residue will be found by solving the proportion  $1.000-0.021 : 1.000 :: 0.116 : x$ , whence  $x = 0.116 \div 0.979 = 0.1185$ .

38. **In practice, we rarely know** beforehand what the coefficient of saturation is, so that we use in a routine way about one volume of immiscible solvent to 4 or 5 of the aqueous solution, shaking continuously two minutes, which is usually quite sufficient to establish equilibrium. We repeat the operation three times and then make a fourth extraction testing a portion of the immiscible fluid to see whether it has taken up anything. If so, we continue the shaking out with fresh portions of the immiscible solvent until the extraction is complete. The shaking out method is not to be recommended, except as a last resort, if the extraction is not practically complete after five repetitions of the shaking out process.

39. **The coefficient of saturation** may be defined from another angle as the fractional portion of the substance left in the original menstruum after shaking it out with an equal volume of the immiscible solvent. This coefficient may be as high as 0.2 if this large proportion of solvent is to be used in the extraction. The following table is self-explanatory. It assumes a coefficient of saturation of 0.1, also that the volume of solution is 50 mls.

Quantity of solvent used in each extraction	Number of extractions to remove 99.9% of substance	Total quantity of solvent
50 mls	4	200 mls
40 mls	6	240 mls
30 mls	7	210 mls
25 mls	8	200 mls
20 mls	8	160 mls
15 mls	9	135 mls
10 mls	10	100 mls

40. **From these data** it appears that there is **economy of solvent in using small** rather than large portions in each extraction. The apparent exception in the case of the largest quantity taken is due to the fact that in this case 4 extractions barely

1/1.04  
2.5  
1.01

suffice to take out 99.9 percent of the substance, while in the other cases the extraction is more complete, although one less washing would not take out quite the full 99.9 percent.

41. Note that there is little or no advantage in using a quantity of solvent greater than 25 mls for each extraction.

42. **In many of the assay processes** described in the following pages, directions are given to use in the successive extractions diminishing quantities of the immiscible solvent. There is advantage in this plan in cases when the quantity of substance to be extracted is large. A good routine procedure is to use for 50 mls or less of the original solution, 20, 15, 15 and 10 mls of the immiscible solvent. When the original solution measures much more than 50 mls, it is advantageous to divide it into two portions and use the same portions of immiscible solvent to shake out the two, one after the other. This really consumes little more time for each extraction than the usual procedure, while there is required very much less of the solvent.

43. **The first consideration** in the selection of an immiscible solvent in any particular case is that of the coefficient of saturation just discussed. This should not be greater than 0.2 if a solvent having so low a coefficient can be found. In a few cases we are obliged to be content with one having a coefficient of 0.3, but the shaking out must needs be tedious.

44. **The solvent most used is chloroform.** Its high specific gravity makes it convenient to use. Its great merit, however, is the avidity with which it takes into solution most alkaloids and many glucosides. It is itself soluble in water only to a very slight extent—less still in saline solutions, while it does not extract from aqueous solutions an appreciable quantity of fixed alkali or alkaline salt. It is less liable than other immiscible solvents to form emulsions. With some alkaloids, notably colchicine, it enters into combination, so that it is necessary to redissolve the alkaloidal residue two or three times in alcohol or ether and

evaporate as long as the residue shows loss of weight. An important point to bear in mind is that chloroform is capable of undergoing a chemical change by which hydrochloric acid is produced, so that solutions of alkaloids in chloroform, unless in contact with an alkaline solution, must not be allowed to stand any length of time before distillation or evaporation. Such solutions should not be exposed to spontaneous evaporation. If the alkaloid is one that is liable to be injured by heat, use a current of warm air to hasten evaporation.

**45. Next in importance to chloroform is ether.** In exceptional cases it is a better solvent than the former. Oftener it is chosen because it fails to dissolve alkaloids that are relatively inert, as in the case of hydrastis. It also leaves behind many resinous and waxy substances which chloroform extracts. As a rule it is to be given preference whenever it is an active solvent for the alkaloid to be extracted, except in the occasional cases where it forms troublesome emulsions. Its disadvantages are that it is lighter than water, so that separations are not as easily made with it, that it is excessively volatile at temperatures above 10°C., and that it dissolves a notable amount of water, and is itself somewhat soluble in water, so that aliquot parts, whether by weight or by volume, are liable to be very inexact.

**46.** Mixtures of ether with chloroform are much used, particularly for the primary solvent in assays of crude drugs. They may be made specifically heavier or lighter than water, the "heavy" mixture made generally in the proportion of 3 volumes of ether to 4 of chloroform, while the "light" mixture consists of 3 to 5 volumes of ether to one of chloroform.

**47. Petroleum ether** (Benzin)<sup>†</sup> is an active solvent for a few alkaloids, e. g. cocaine, and when this is the case is well adapted for use in the shaking out process. Its advantages are practically complete insolubility in water, and limited range of solvent action, so that the crude extracted alkaloid is compa-

<sup>†</sup>For assay work petroleum ether should be redistilled, only that portion being retained which comes over below 70° C.



encl 2 +

ratively free from impurities. Its drawbacks are (1) indefiniteness of composition with a corresponding variability in solvent action and in volatility, (2) a pronounced disposition to form emulsions, (3) a low specific gravity. A mixture of petroleum ether with chloroform combines the good qualities of the two constituents, and its practical use may well be subject for experiment. One use made of petroleum ether is for throwing out of solution in chloroform or other solvent certain substances which we wish to eliminate, or occasionally the substance we wish to purify.

48. **Other solvents** such as amyl alcohol, isobutyl alcohol, amyl acetate, ethyl acetate, carbon tetrachloride, ethyl bromide, mixtures of chloroform with ethyl alcohol, methyl alcohol, etc., have their special limited spheres of usefulness, which need not be particularized here.

EXCEPTIONAL BEHAVIOR OF CERTAIN ALKALOIDS

49. **The rule is that immiscible solvents remove practically no alkaloid from an acid solution**, while acids in aqueous solution extract all alkaloids from solutions in immiscible solvents. There are, however, important exceptions to the rule as follows:

50. (1) From a solution strongly acidulated with hydrochloric acid, chloroform removes caffeine and papaverine, and a notable quantity also of strychnine, the quantity in the case of strychnine (not of caffeine) increasing with the amount of acid present. (2) Other bases that are taken up in small quantities, particularly when the proportion of acid is small, are aconitine, brucine, cocaine, codeine, narceine, narcotine, pelletierine, thebaine and veratrine. Hence if hydrochloric acid must be used to acidify the solution a good excess should be added in the case of the alkaloids just named, while in the case of strychnine, only sufficient should be used to give a distinct acid reaction. Atropine, coniine, daturine, emetine, hyoscyamine, morphine, nicotine, quinine and sparteine are practically not at all extracted by chloroform from solutions containing free hydrochloric acid. (3) From solutions containing free sulphuric acid, caffeine and

papaverine are removed as free bases, and narcotine partly as base, partly as sulphate. Appreciable quantities also of aconitine, brucine, emetine, narceine, pelletierine, strychnine and veratrine are extracted, but the amount is negligible if the solution contains as much as one percent of acid. (4) From neutral solutions saturated with sodium chloride, aconitine and narcotine are wholly removed by chloroform. (Atropine and quinine are also partially removed.)

51. Note that extraction of morphine, narceine and the glucoside strophanthin from alkaline solutions (sodium carbonate) by a mixture of chloroform and alcohol, is greatly assisted by saturating the solutions with sodium chloride.

52. (5) From a solution acidified with tartaric acid, traces of brucine and strychnine are removed by chloroform.

53. (6) From a solution acidified with hydrochloric acid, ether extracts in weighable amounts only caffeine and narcotine. Traces of aconitine, emetine and narceine are taken up.

54. (7) From a solution containing free sulphuric acid, ether takes out only caffeine in notable quantity.

55. (8) Ether does not remove brucine or strychnine from solutions containing free tartaric acid, but it extracts much caffeine from one acidified with oxalic acid.

56. **The Perforator method** (the principle of continuous extraction applied to liquids). A simple and effective mode of extracting alkaloids from an aqueous solution is by aid of the apparatus devised by Hulsebosch, fantastically called a **perforator**. It consists of a flask in which the immiscible solvent can be boiled, provided with a condenser which delivers the distilled fluid drop by drop into the fluid from which the alkaloid is to be extracted, and through which it is made to pass on its way back to the distilling flask. The alkaloid is set free by adding to the aqueous solution a sufficient quantity of water of ammonia, or other suitable alkali, taking care that the solution is so dilute that the precipitated alkaloid does not agglomerate so as to resist the action of the

immiscible solvent. The apparatus once set up and charged, its operation goes on continuously without attention until the whole of the alkaloid is dissolved and transferred to the flask.

In order to obtain the alkaloid in a high state of purity, the acid solution is first treated with petroleum ether by aid of the "perforator" to remove traces of chlorophyll, etc. that may be present. As a rule, however, it is sufficient to extract the alkaloid without such preliminary treatment, evaporate off the solvent and determine the alkaloid in the residue by alkalimetric titration in the usual way.

## DETERMINATION OF ALKALOIDS BY ALKALIMETRY

57. The alkaloids extracted by the methods described in the foregoing paragraphs carry more or less impurity, so that it is only in exceptional cases that a standard is based on the weight of such alkaloid. The determination may be made gravimetrically by dissolving the alkaloid and precipitating it as an insoluble compound of definite composition, but in the majority of cases some volumetric method is resorted to, a determination of the acid-saturating power of the alkaloid being generally the quickest way of reaching a result, and in most cases quite as satisfactory as any.

**58. Precautions in volumetric determinations to ensure accurate results.** 1. All volumetric apparatus such as measuring flasks, pipettes, burettes and graduated cylinders must be tested as to accuracy of graduation before it is used. This is done with the greatest precision, by filling with mercury at standard temperature and weighing, but this is impracticable when the weight of the mercury exceeds 500 gm. Distilled water answers the purpose sufficiently well. It should be boiled to expel absorbed gases. There will be found in the appendix of the U. S. P. tables giving the apparent weight of 100 mls of distilled water at temperatures ranging from 15° to 35° C., under average conditions of barometric pressure and humidity.

**59. The necessity for testing each piece of apparatus,** even when received from a reliable manufacturer, arises from the fact that standard temperatures are taken variously as 60° F. (in Great Britain), 59° F. (15° C.), (in most countries where the centigrade thermometer is used), 20° C. (U. S. Bureau of Standards) and 25° C. (U.S.P.). The standard is not always stated on the piece of apparatus as it should be. More important than this is the fact that the Mohr liter, holding not 1000 cubic centimeters (mils), but

1000 gm. of distilled water at standard temperature is still in use, and as a rule without any mark indicating that the standard is not the official one.

60. **In making these tests (with water)** it is necessary, as it is in all volumetric work, to make sure that the apparatus is perfectly free from any suggestion of greasiness. Always take notice in using a pipette or burette whether the liquid flows freely and evenly down the surface of the glass. If it fails to do this, the piece of apparatus must be emptied and cleaned with a hot solution of sodium hydroxide. If this fails to remove the apparent greasiness, try strong sulphuric acid, or preferably a solution of potassium dichromate in 85% sulphuric acid. This rarely fails to remove the film, which may prove however to be most readily soluble in some such solvent as alcohol, petroleum ether or chloroform.

61. **Testing of burettes and graduated pipettes**—unless these can be procured of certified accuracy—is particularly important. The exactness of the graduations throughout the length of the scale must be tested. This can be done by drawing off successive 5 mil (or 2 mil) portions of water measured very accurately, and weighing them—a laborious operation. A simpler plan is to fill the burette to the lowest graduation with water, then introduce with a measuring pipette successive 5 mil portions of water, taking an accurate reading each time of the total volume. Even if the quantity delivered by the pipette is not an accurate 5 mils, the method shows whether or not the graduation is uniform, provided no portion of the delivery tube is distensible.

62. **A measuring pipette** must always be given time for complete drainage, not less than one minute when less than 40 cm. long, the time allowed to be increased proportionately if it exceed that length. Care must be taken in accurate work that the temperature of standard solutions when they are used, does not vary more than 2° C. from that at which they were standardized, but it is not essential that this shall be 25° in accordance with the U. S. P. The temperature to be chosen should be near the average air

temperature of the laboratory so that 20° C. is generally to be preferred to 25°.

63. It is not necessary to repeat here the instructions contained in the chapter on volumetric solutions in U. S. P. IX, pp. 555-7, which should be familiar to everyone who attempts standardization of pharmacopoeial drugs or preparations.

**64. For rapid every-day approximate determinations,** the graduated pipette is to be preferred to the burette. Readings must be taken with great care, preferably with the aid of a lens, and always with the pipette held in a strictly vertical position.

**65. Standard solutions are sometimes made up by weight instead of by volume.** The advantages of this plan are obvious, at least in all cases where there is no question about the end point. The standard solution is placed in a dropping bottle and weighed before and after the titration. Care is needed of course not to overrun the end point, but on the other hand the successive portions added towards the end of the titration may be only a fraction of a drop, being delivered by aid of a glass rod.

#### ALKALIMETRIC TITRATION OF ALKALOIDS

66. With a few exceptions, alkaloids may be determined with reasonable exactness by alkalimetric titration. A few alkaloids like sparteine have such pronounced alkalinity that phenolphthalein may be used as indicator. As a rule, however, only those indicators which are particularly sensitive to alkalis can be used in the titration of alkaloids. Even these are not affected by caffeine, berberine or colchicine, and respond only feebly or imperfectly in the case of some other alkaloids, notably hydrastine, emetine and even the cinchona alkaloids. In general a reasonably sharp end point may be found with iodeosin, cochineal, methyl red or haematoxylin. The first of these is used in ethereal solution from which it passes into the aqueous solution containing the alkaloid as soon as the slightest excess of alkali hydroxide is present (provided the solutions are shaken together vigorously). A similar procedure is followed in the

case of uranin and sometimes of haematoxylin. The other indicators are added directly to the solution to be titrated, which should be as nearly as possible free from a color of its own.

## CHOICE OF INDICATORS

67. **C. Kippenberger** several years ago made a special study of the choice of indicators for use with different alkaloids.\* The alkaloid is dissolved in a measured excess of decinormal acid, this excess being determined by titration with a highly dilute volumetric alkali (sodium, potassium, calcium or barium hydroxide). The indicators studied by Kippenberger included azolitmin, (or purified litmus), cochineal, haematoxylin, iodeosin, lacmoid, methyl orange and uranin. The results of his numerous experiments are summarized in the following table which gives for each alkaloid named, 1st the indicator or indicators found quite satisfactory, and 2nd, in parenthesis, those available as second choice. The prominence given to azolitmin and lacmoid is suggestive. Methyl red is not mentioned, not having then attracted attention as an indicator.

## 68. Table of Preferable Indicators

ALKALOID	INDICATORS
Aconitine	Azolitmin
Atropine	Uranin, Lacmoid (Methyl Orange)
Brucine	Cochineal (Uranin, Lacmoid)
Cocaine	Lacmoid
Codeine	Iodeosin, Lacmoid (Azolitmin)
Coniine	Iodeosin, Cochineal, Lacmoid (Methyl Orange, Azolitmin)
Emetine	Iodeosin, Cochineal (Azolitmin, Uranin, Haematoxylin, Lacmoid)
Morphine	Lacmoid (Iodeosin)
Narcotine	Lacmoid (Methyl Orange)
Nicotine	Lacmoid (Iodeosin, Uranin, Cochineal)
Papaverine	Lacmoid
Pelletierine	Cochineal (Iodeosin, Lacmoid)
Quinine	Azolitmin, Haematoxylin

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\*Zeitschr. f. anal. Chem. XXXIX, 201.

Sparteine	Haematoxylin (Uranin)
Strychnine	Azolitmin (Uranin, Lacmoid)
Thebaine	Iodeosin, Cochineal (Haematoxylin)
Veratrine	Lacmoid

69. We miss in this list particularly the alkaloids Cinchonine, Cinchonidine, Quinidine, Hydrastine, Physostigmine and Pilocarpine. Of these the first three resemble quinine. For the mixed alkaloids of cinchona bark, in the judgment of the writer, none of the common indicators are to be considered except haematoxylin, methyl red and perhaps azolitmin and uranin, see (238 and 239). Hydrastine is very feebly alkaline so that with methyl orange the end point of the titration is indistinct. The alkaloid is so nearly insoluble that a titration may be made without any indicator, the end point being indicated by the appearance of a distinct turbidity in the solution. See (320).

70. **Routine method of determining alkaloids by alkalimetric titration.** The alkaloidal residue to be titrated is dissolved in 5 mls of strictly neutral alcohol. To the solution is added first a few drops of the indicator selected, then an accurately measured quantity of the volumetric acid, making sure that there is a distinct excess of this. If the crude alkaloid has been weighed, as it should be, the theoretical amount is determined by dividing its weight by the titration factor of the alkaloid in question, multiplying the quotient by 1.5 for good measure. Add to the solution about 15 mls of recently boiled distilled water of assured neutrality, and then titrate the excess of acid, best with lime water, using for this purpose a 10 mil pipette graduated to twentieths of a mil, rather than a burette. Make a memorandum of the quantity of lime water required. Now measure into a suitable flask the same quantity of volumetric acid as was used in the first experiment. Add indicator and distilled water as before and titrate, to ascertain the exact strength of the lime water used. Subtract the result of the first titration (A) from that of the second (B). The difference (C) is the measure in lime water units of the amount of alkaloid present.



The value of the unit (D) is found by dividing the number of mls of volumetric acid taken by the number of mls of lime water (B) consumed in the titration, this quotient approximating 0.4. Multiply C by D to find the measure of alkaloid in terms of decinormal acid, and finally multiply this product by the titration factor of the particular alkaloid present for the weight in grams of such alkaloid. Representing this factor by  $f$  and the weight of this alkaloid by  $x$ , the mathematical formula is  $x = fCD$ .

**71. Example.** The alkaloidal residue under examination is that from 10 grammes of belladonna root. Its weight is 0.0625 gm. If it were pure atropine or hyoscyamine, it would require for neutralization more than 2 mls of decinormal acid ( $0.0625 \div .02892 = 2.17+$ ). We use therefore to dissolve it 3 mls of our standard acid. Titration of the solution consumes 2.60 mls (A) of lime water, while titration of 3 mls of the acid consumes 7.28 mls (B). The difference ( $B - A = C$ )  $7.28 - 2.60 = 4.68$  is the measure in terms of lime water of the alkaloid present, and this multiplied by the factor ( $3 \div B = D$ ) 0.4121 gives the number of mls of decinormal acid equivalent to the alkaloid dissolved ( $C \times D = 1.929$ ). Multiply this by the titration factor for atropine to get the weight of the alkaloid, estimated as atropine ( $1.929 \times 0.02892 = 0.05579$ ). Since this was the yield from 10 gm. of drug, the percentage of alkaloid in the belladonna root is 0.5579.

**72. Lime water for use as a volumetric alkali** has the following advantages. I. It remains always caustic, so that the end point in a titration is always as sharp as the nature of the case permits. As the solution absorbs carbon dioxide from the air, the calcium carbonate formed separates as a coherent incrustation on the sides and bottom of the container, the reagent always remaining quite transparent and free from suspended sediment. II. As long as the stock solution stands over an excess of calcium hydroxide, it remains practically constant in strength, whatever the room temperature. A portion of the

solution can generally be decanted perfectly clear when required for use. Should it show any turbidity, it can be filtered, taking care to run through the filter first a quantity of the solution, which is to be rejected or returned to the stock bottle. Any portion of the reagent remaining after the titration is carried out can also be returned to the stock bottle, although if there is occasion to make frequent titrations, the reagent may be kept a week or two in a well corked bottle (which should be nearly full) with very little deterioration—its exact strength being determined every time it is used in the routine of the titration. III. Since it varies but little in strength, it serves as a check on the strength of the standard acid, upon which the correctness of the results reached depends. Hydrochloric acid is chosen in preference to sulphuric partly because the monobasic acid may be expected to give a sharper end point, partly on account of the ready solubility of the calcium chloride formed, as compared with calcium sulphate.

73. **Direct titration of alkaloidal residues** is practised by some, the indicator chosen being usually iodeosin. Twenty mls of ether and 100 mls of water, with 5 drops of iodeosin solution, are placed in a stoppered flask and the mixture brought to exact neutrality by addition of highly dilute alkali or acid. The ethereal solution containing the alkaloid is added and its alkalinity determined by direct titration with fiftieth- or hundredth-normal hydrochloric acid. Haematoxylin is sometimes used as the indicator, the alkaloidal residue being dissolved in neutral alcohol and the solution diluted with (neutral) distilled water. An indicator which is used in a similar manner is uranin, the sodium salt of fluorescein. As long as free alkaloid is present, the aqueous fluid shows a characteristic green fluorescence, which disappears when the slightest excess of acid is added. The titration is, however, generally made residually, excess of acid being titrated with tenth-normal alkali to the appearance (after shaking) of a permanent fluorescence in the aqueous fluid.

#### 74. Improvement of the alkalimetric method

**for determining alkaloids.** Harry M. Gordin\* (1899) proposed an improvement in the alkalimetric titration of alkaloids whereby it was believed that the difficulty due to feebleness of alkaline character was overcome. The alkaloid is dissolved in a measured excess of volumetric acid (hydrochloric or sulphuric) and the alkaloid is then precipitated with some reagent which will form a definite compound with it, taking care that the reagent contains no free acid or alkali. The reagents recommended were the potassio-mercuric iodide (Mayer's reagent) and the decinormal iodine volumetric solution (Wagner's reagent).† In either case, the alkaloid theoretically carries with it as it is precipitated exactly an equivalent weight of the acid, leaving the remainder free so that it can be readily titrated with **any indicator, even phenolphthalein.** The solution after precipitation is brought to a definite volume, nearly all of the necessary water being added before the precipitation is made, so that a minimum amount of free acid shall be occluded in the precipitate. The solution is filtered, the first third of the filtrate being rejected, and an aliquot part is then taken for the titration, in which a twentieth-normal solution of potassium or sodium hydroxide is employed. The theory is highly plausible but in practice the method has proved somewhat disappointing as to the exactness of the results reached. It is, however, worthy of further study, at least in its application to such alkaloids as hydrastine and the combined ether-soluble alkaloids of ipecac.

**75. Christensen has proposed an iodometric method** of determining the residual acid in titration of alkaloidal residues, similar to Kjeldahl's iodometric method of determining free ammonia. The alkaloid is dissolved in a measured excess of tenth-normal acid

\*Pharm. Archiv. 1899, 2, 213-8; Ber. 1899, 32, 287. See also Pharm. Archiv. 1901, 214-31.

†When Wagner's reagent is used as the precipitant it is necessary to add to the solution before titrating it a sufficient quantity of sodium thiosulphate to decolorize it, taking care that the salt is strictly neutral. The Wagner's reagent itself must also be proved to be neutral.

and to the solution a neutral solution of potassium iodide together with a solution of potassium iodate in equivalent quantity is added, when the excess of free acid present will liberate an equivalent of iodine which is then to be titrated with tenth-normal sodium thiosulphate V. S. Subtract the number of mls of thiosulphate solution used from the number of mls of tenth-normal acid taken to dissolve the alkaloid and multiply by the alkaloidal factor (e. g. for atropine by 0.02892) to find the quantity of alkaloid present. The alkaloidal residue in this case is to be dissolved in tenth-normal sulphuric acid solution. With most alkaloids good results are obtained.

**76. Volumetric determination of the alkaloid as hydrochloride.** Another procedure may be adopted for determination of alkaloids which form strong combinations with acids, and which are not easily decomposed by excess of acid. The alkaloid in ethereal or alcoholic solution may be supersaturated with an ethereal solution of hydrochloric acid, evaporated by a gentle heat, taken up once or twice with dehydrated alcohol or a mixture of alcohol and ether and dried again to drive off completely excess of acid, and the residue dissolved in distilled water and titrated with a hundredth-normal solution of silver nitrate, using potassium neutral chromate as indicator, to determine the chlorine and hence indirectly the quantity of alkaloid present. The titration factor (except in the rare case of an acid salt) will be the same as that for an alkalimetric determination. In any case it is easily deducible from the molecular weight of the alkaloid.

**77.** Perhaps an excess of silver nitrate may be used and a residual titration made by the new method of Louis Schneider,\* which differs from the Volhard method in using potassium iodide in place of potassium sulphocyanate, employing for indicator a solution of palladious nitrate.

**78. A modification** of this method has been found by E. Elvove† to give very satisfactory results with a

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\*Journ. Am. Chem. Soc., April 1918, 583-93.

†Journ. Am. Chem. Soc., 1909, 132-9. See also Bull. 54. Hyg. Lab. U. S. Pub. Health Service, Washington, D. C.

large number of alkaloids, including narcotine, hydrastine and the cinchona alkaloids, all of which are difficult subjects for alkalimetric titration. His procedure is as follows: Dissolve 0.2 gm. of the sample (e. g. crude alkaloid obtained in an assay) in 5 mls of diluted hydrochloric acid (10%) and evaporate to dryness on a water bath. Dissolve the residue in 5 mls of strong alcohol and again evaporate to dryness on the water bath. Repeat the treatment with alcohol to ensure complete elimination of free hydrochloric acid. Dissolve the residue in 10 mls of distilled water, add phenolphthalein indicator and titrate the solution with tenth-normal, or twenty-fifth-normal alkali (lime water may be used advantageously).

79. Filter the solution and wash the precipitated alkaloid with distilled water (a minimum quantity) until free from chloride. Make up the filtrate including washings to about 70 mls, add 5 mls of diluted nitric acid (10%) and then a moderate excess of tenth-normal silver nitrate (10 or 15 percent more than the amount of tenth-normal alkali required in the neutralization). Make up to 100 mls with distilled water and filter through a dry filter. To 50 mls of the filtrate add 1 ml of a 10 percent solution of ferric ammonium sulphate, and titrate the excess of silver with tenth-normal potassium thiocyanate. Multiply the number of mls of thiocyanate solution by two and subtract from the quantity of silver nitrate solution taken. The remainder multiplied by one-tenth of the molecular weight of the alkaloid in question is the weight in milligrams of the alkaloid in the sample taken.

80. In practise it is found that the presence of the alkaloid in the solution embarrasses the titration, even when no coloration is caused by the nitric acid (brucine, morphine). Hence it is best to extract the alkaloid in the outset by shaking out with an alkali (chloride free) and an immiscible solvent, and then add the silver nitrate to the residual solution. The alternative plan is to mix the hydrochloride with sodium carbonate and a little potassium nitrate (both reagents chloride-free) and ignite moderately.

The residue is to be extracted with hot water, and the chlorine it contains is to be determined by Volhard's or Schneider's titration method.

**81. The lead subacetate method of assay.** In cases where the alkaloid does not show an alkaline reaction with indicators and in general where the determination is to be gravimetric, the most useful general process is one in which lead subacetate is employed to remove organic acids and other non alkaloidal compounds from a solution before shaking out the alkaloids. The alkaloids having been brought into a moderately dilute neutral aqueous solution (which may contain also a little alcohol), a solution of lead subacetate, the liquor plumbi subacetatis of the U. S. Pharmacopœia, is added in slight excess—about  $\frac{1}{2}$  mil to each gm. of crude drug is generally sufficient—an excess being generally easily recognized by the sweetish taste of a drop of the mixture. The solution is brought to a definite volume, e. g. 100 mls from 5 or from 10 grammes of drug, and after standing 15 to 20 minutes is filtered through a dry filter. To the filtrate is added about 1 gm. of powdered sodium phosphate, to precipitate excess of lead. The solution is again filtered through a dry filter, making sure that the sodium phosphate is in excess by adding to the first portion of the filtrate a little more sodium phosphate.

**81½.** Shake out (preferably with chloroform) the alkaloid from an aliquot portion of the filtrate, after adding water of ammonia if necessary to set the alkaloid free. Evaporate off the solvent, take up the residue with 2 mls of alcohol and evaporate again, repeating this once more to drive off every trace of chloroform, dry and weigh. If the determination is to be gravimetric, purify the alkaloid, which may contain fatty matter, by dissolving in highly dilute acid or, in case of caffeine or colchicine, in warm water. If a clear solution results, with no residue left adhering to the beaker, the alkaloid may be considered to be pure; if this is not the case, put into the beaker about 1 gm. of hard paraffin, heat until the paraffin melts

and stir well until the insoluble residue has been taken up by the paraffin. Let the mixture stand until the paraffin solidifies, remove this and melt it with 5 mls of water, cool, separate the paraffin and add the water to the solution in the beaker. From this solution (filtered) extract the purified alkaloid by rendering alkaline (if necessary) and shaking out with chloroform in the usual manner. Unless the alkaloid is caffeine or colchicine, preferably determine it by alkalimetric titration without treating with paraffin.

### 82. Isolation of alkaloids by absorbents.

Certain substances such as charcoal have the property of removing from solutions some of their constituents by selective adsorption. Filtering papers possess more or less of this property, and in a greater degree the various substances employed to aid filtration, such as powdered pumice. One must bear this in mind always in taking an aliquot part of a filtered solution. The precaution should always be taken to reject the first portion—perhaps 25 per cent—of the filtrate, as likely to have lost some portion of its constituents, taking the aliquot from the portion of the solution which next passes through the filter.

### 83. Hydrous Aluminum Silicate (Lloyd's Reagent) as a precipitant for alkaloids.

J. U. Lloyd in 1914 announced the discovery that certain forms of aluminum silicate, particularly that obtained from fuller's earth, have the property of removing almost instantaneously from neutral or acid solutions, a number of substances including the active principles of many vegetable drugs. The reagent seems to enter into physical or chemical combination with alkaloids, glucosides and other compounds, such combinations being practically insoluble in water, and so free from taste. They are, however, capable of producing the toxic and therapeutic effects of the alkaloids, etc., which they contain. The quantity of the reagent required to remove 1 part of an alkaloidal salt from solution varies from 6 parts (for morphine sulphate) to 14 parts (for cocaine hydrochloride). It was at

first thought that alkaloids could be isolated quantitatively by means of the reagent, the combination being broken up by the action of solvents, but this expectation has been realized only to a very limited extent. Future research may develop, however, accurate assay methods based on this principle for some of the alkaloids.

**84. The periodide assay of Gordin and Prescott.\*** Precipitates of definite composition are formed when certain alkaloids are treated with an excess of Wagner's reagent. The neutral solution of the alkaloid is poured with constant stirring into a measured volume of the reagent (tenth-normal iodine V. S. of the U. S. P. IX) to which has been added a little diluted hydrochloric acid, and the solution is brought to a definite volume. It is then shaken vigorously until the supernatant liquid is perfectly transparent. It is essential, except in the case of the alkaloid morphine, that the excess of iodine shall be sufficient to give the solution a dark red color. If this is not the case, a new experiment must be made, using a larger quantity of the iodine solution. Finally an aliquot of the solution is pipetted off, and titrated with tenth-normal sodium thiosulphate V. S. Subtracting the quantity of thiosulphate solution from that of the iodine solution taken, we have the quantity of iodine volumetric solution consumed. Multiply this by the appropriate factor to find the actual quantity of alkaloid present. The following are the factors in question:

Aconitine.....	0.010757
Atropine.....	0.003615
Brucine.....	0.006571
Caffeine.....	0.004853
Morphine.....	0.009505
Strychnine.....	0.005570

**85.** The method seems to give concordant results in the cases of morphine and caffeine, but only if strict attention is given to every detail of the assay. With the other alkaloids results have not been found wholly satisfactory in practice, yet the method is worthy of further study.

\*Journ. Am. Chem. Soc., May 1898, 329; Proc. Am. Pharm. Assoc. 1898, 355, and 1899, 271.



86. **The several alkaloids can be recovered** from the polyiodide precipitates by treatment with sulphurous acid or an alkaline sulphite and dilute sulphuric acid, or an alkaline thiosulphate (hyposulphite), then adding an alkali and shaking out with an immiscible solvent.

87. **Determination of alkaloids by use of Mayer's Reagent.** In the earlier days of alkaloidal assays much use was made of Mayer's reagent\* as a quantitative precipitant of the alkaloids. The procedure was a very simple one. An aqueous solution was prepared containing about 0.5 percent of the alkaloid. This was made distinctly acid with diluted hydrochloric or sulphuric acid. Alcohol and acetic acid were not allowed to be present. Mayer's reagent was added from a burette as long as it produced any cloudiness in the solution and then by use of an empirical factor the quantity of alkaloid corresponding with the amount of volumetric solution used was calculated. In the case of a few alkaloids the end point of the titration was quite sharp, although the necessity of repeated filtration of the solution rendered the operation somewhat tedious. In most cases, however, it was difficult to fix exactly the end point since precipitation was not complete until a considerable excess of the reagent had been added.

88. This difficulty was in a measure overcome by the expedient proposed by the present author† of adding at once a decided excess of the reagent, filtering the solution and adding to the filtrate a definite quantity of a strychnine solution, more than sufficient to combine with the excess of Mayer's reagent present. Finally the excess of strychnine was determined by titration with Mayer's reagent, the end point in the case of this alkaloid being quite sharp.

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\*Mercuric potassium iodide T. S. of U. S. P. IX. Dissolve 1.358 gm. of mercuric chloride in 60 mls of distilled water, and 5 gm. of potassium iodide in 10 mls of distilled water, mix the solutions and add distilled water to make 100 mls. The reagent is one-twentieth normal.

†Lyons, *Assay of Drugs* (1898) p. 66.

89. **Dr. Gunnar Heikel** more recently devised a simple expedient for determining the excess of Mayer's reagent so that results of satisfactory precision can be reached by the improved titration in the case of a number of alkaloids.\* Dr. Heikel adds in the first place an excess of Mayer's reagent, filters the solution, and adds to an aliquot part of the filtrate an excess of a standardized solution of potassium cyanide, by which the mercury is converted into cyanide. Ammonia (10 mls of 10 percent) is then added with a few drops of solution of potassium iodide, and the excess of potassium cyanide is determined by titration with twentieth normal silver nitrate, to the appearance of permanent turbidity.

90. **Method of titration with Mayer's reagent.** Put the solution (10 to 20 mls) to be titrated, which should contain about 0.5 per cent of alkaloid, into a small beaker and add from a burette with stirring about one half the quantity of Mayer's reagent which should be required. Filter through a dry filter which will hold the whole of the fluid at the close of the titration, but not much more. When the fluid has all run through, add to the clear filtrate a drop of the reagent, judge from the density of the precipitate formed how much more of the reagent will probably be required, add a little less than that quantity and return the fluid to the filter. If it does not run through clear, return it once or twice to the filter. Thereafter add the reagent two or three drops at a time according to effect, until a single drop fails to produce a permanent cloudiness in the filtrate.

91. Time will be saved by taking three or four portions of the alkaloidal solution and adding to the first one-half, to the second two-thirds, to the third three-fourths, to the fourth the full quantity that should be required. Filter all and add to each of the several filtrates a drop of Mayer's reagent, continuing the titration with the one in which the faintest turbidity is produced.

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\*Chem. Ztg., 1908, 32, 1149-1151, 1162-1163, 1186-1187, 1212-1213.

**92. Recovery of the alkaloid from the precipitate** by Mayer's reagent may be effected according to Prescott\* by triturating the precipitate with a solution of stannous chloride, adding potassium hydroxide to strong alkaline reaction and extracting with the appropriate immiscible solvent. If potassium carbonate be substituted for the caustic alkali, strong alcohol may be employed for the extraction. Another plan suggested by Prescott is to dissolve the precipitate in alcohol, adding acid if necessary, and precipitating the mercury with hydrogen sulphide gas. The filtrate can be freed from iodine, if this be desired, after expelling the excess of hydrogen sulphide, by adding excess of silver nitrate, filtering, adding hydrochloric acid to remove excess of silver and filtering again, the alkaloid being now in the form of a hydrochloride or nitrate.

**93. Simpler than the foregoing** is the plan of T. B. Groves,† who directs to wash the precipitate slightly, suspend it in water and treat with an excess of silver nitrate (or of lead acetate). The filtered solution may be freed from excess of silver by a chloride (or from lead by a sulphate), rendered alkaline and shaken out with an appropriate immiscible solvent.

**93½. Gravimetric use of Mayer's reagent.‡** Results of practical value may be reached by precipitating a solution of alkaloid (approximately 1:200) under prescribed conditions with a distinct excess of Mayer's reagent, collecting the precipitate on a tared filter, washing it with a little distilled water, drying it to constant weight at 100° C. and weighing it. Multiply the weight by an empirical factor to find approximately the weight of the alkaloid. The figures of the following table are subject to emendation, but may serve a useful purpose.

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\*Organic Analysis, 1887, p. 46.

†Lyous, Assay of Drugs pp. 65-67.

‡Pharm. Journ. Trans., (II), vol. 6, 275.

## GRAVIMETRIC FACTORS

Name of Alkaloid	Empirical Factor	Name of Alkaloid	Empirical Factor
Aconitine.....	0.540	Colchicine.....	0.625
Atropine.....	0.455	Alkaloids of Ipecac...	0.390
Berberine.....	0.510	Hydrastine.....	0.488
Brucine.....	0.485	Morphine.....	0.500
Cinchonidine.....	0.285	Quinine.....	0.311
Cinchonine.....	0.295	Strychnine.....	0.374

**94. Determination of alkaloids by Silicotungstic Acid.** Of all the reagents which precipitate alkaloids the one best suited for quantitative determinations is silicotungstic acid.\* Not only are the precipitates which it forms quite definite in composition and generally at once stable and exceedingly insoluble, but they yield by ignition a residue which may be weighed with great precision. The limit of sensitiveness of the reaction is given as: for morphine, 1:16,000, for theobromine, 1:18,000, for nicotine, 1:20,000; for narceine, 1:30,000, for codeine, 1:40,000, for atropine and caffeine 1:50,000, for aconitine, 1:80,000, for veratrine, 1:130,000, for brucine, 1:150,000, for cocaine, narcotine and strychnine, 1:200,000; for quinine, quinidine, cinchonine and cinchonidine, 1:500,000.

**95.** The several alkaloids may be extracted in crude form by the usual assay methods, dissolved in diluted acid, precipitated by silicotungstic acid, the precipitate collected, washed, dried, ignited and weighed and the weight multiplied by the appropriate factor, to find the weight of pure alkaloid present. Otherwise an aqueous solution may be prepared representing a definite quantity of drug. This may be precipitated by silicotungstic acid, the precipitate treated with an alkali hydroxide or with magnesia to set free the alkaloid, which may then be shaken out with an appropriate solvent.

**96.** Determination of alkaloids by precipitation with **potassium-bismuth iodide** (Dragendorff's re-

\*G. Bertrand, Compt. Rend., 1899, 128, 742-5.

agent). The reagent is prepared as follows: Dissolve 8.0 gm. of bismuth subnitrate in 200 mls of nitric acid, sp. gr. 1.18, pour this slowly with constant agitation into a solution of 272 gm. of potassium iodide in 275 mls of water. Chill the mixture four hours in an ice-water bath, then decant the fluid carefully from the crystals of potassium nitrate, and bring the volume of the solution to 1 liter. (J. C. Thresh prepares the reagent by adding to 96 mls of "solution of bismuth citrate, B. P." (solution of bismuth and ammonium citrate B. P.) 6 mls of hydrochloric acid, sp. gr. 1.16 and 7 gm. of potassium iodide.)

97. Many alkaloids are precipitated very completely by this reagent, which has been employed by H. Thoms\* for the quantitative determination of atropine and strychnine, and by D. Jonescu† for the estimation of quinine, caffeine and antipyrine.\*\* The precipitate formed by adding the reagent to an acid solution of the alkaloid is collected on a filter and washed twice with 10 percent sulphuric acid (5 mls). The filter containing the moist precipitate is transferred to a glass-stoppered flask and treated with 0.3 gm. of sodium sulphite and 15 mls of a 15 percent solution of sodium hydroxide. The mixture is shaken vigorously for half an hour or more to decompose the precipitate; 8 gm. of sodium chloride are added, followed by ether or other appropriate immiscible solvent and the alkaloid is shaken out and determined in the usual manner, most commonly by alkalimetric titration.

#### 98. Phosphomolybdic acid (Sonnenschein's)

\*Journ. de Pharm. et Chim., 1905, XXI, 605.

†Ber. Deut. Pharm. Ges., 16, 130-2.

\*\*Precipitates are produced according to J. C. Thresh in solutions of Caffeine, 1:3,000; of Berberine, 1:6,000; of Apomorphine, 1:12,500; of Codeine, 1:17,500; of Morphine and of Narceine, 1:20,000; of Atropine, 1:25,000; of Brucine and of Aconitine, 1:40,000; of Narcotine, 1:50,000; of Cinchonidine, 1:125,000 of Quinidine, 1:150,000; of Quinine, 1:200,000; of Strychnine, 1:250,000.

**reagent\*)** removes most alkaloids very completely from their aqueous solutions, e. g. from an infusion of an alkaloidal drug which has been previously precipitated with a solution of lead subacetate. The reagent precipitates also ammonia and some other non-alkaloidal compounds. The alkaloid can be recovered from the precipitate by mixing it in a moist condition with potassium or sodium carbonate and extracting the pasty mixture with strong alcohol or with an immiscible solvent.

99. Very similar in behavior to the foregoing is **Scheibler's reagent**, phosphotungstic acid, prepared by dissolving sodium tungstate, 100 gm. and sodium phosphate, crystallized, 80 gm. in 500 mls of water, and adding nitric acid to acid reaction. The alkaloids can be recovered from the precipitate after washing it with water containing phosphotungstic acid and ammonia by mixing it with barium or calcium hydroxide and extracting with chloroform (or in case of a volatile alkaloid by distillation).

100. A limited use is made of **Picric Acid** for quantitative precipitation of alkaloids. A saturated aqueous solution is used. The precipitates are crystalline and of definite composition. In the case of the cinchona alkaloids, the precipitated picrates are simply washed with cold water, dried and weighed. More exact results may be expected from extraction of the alkaloid by treating the precipitate with an alkali and the appropriate solvent. Nicotine, brucine and berberine have also been determined by the use of this reagent, but many alkaloids are precipitated only from concentrated solutions. It may be particularly noted that morphine, caffeine and coniine are not precipitated in solutions acidulated with sulphuric acid.

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\*Prepared by adding to a warm solution of sodium phosphate, acidulated with nitric acid, an excess of ammonium molybdate solution, washing the separated yellow precipitate with water acidulated with nitric acid and dissolving it in a hot solution of sodium carbonate. The solution is evaporated to dryness, the residue ignited at a low red heat, moistened with nitric acid and again ignited and finally dissolved in ten times its weight of a mixture of one volume of nitric acid, sp. gr. 1.42 with 9 volumes of water.

101. **Picrolonic Acid** (dinitrophenylmethylpyrazolone) is, however, much better suited to such use, since it precipitates the alkaloids much more completely. Its application to the assay particularly of *nux vomica*, *hydrastis* and *pilocarpus*, has been recommended by H. Matthes and O. Rammstedt,\* and its use is likely to be still further extended since it precipitates quantitatively morphine, codeine, cotarnine and other alkaloids.† The reagent is generally employed in alcoholic solution, 0.0264 gm. in each mil (tenth-normal). The precipitated picrolonates may be weighed as such, or they may be decomposed by warm diluted sulphuric acid, and the liberated picrolonic acid shaken out with acetic ether, leaving the pure alkaloid in the form of sulphate in the acid solution.

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\*Arch. de Pharm., 1907, 112, 132.

†Chem. Centralbl., 1907, (II), No. 16.

## ROUTINE OF ASSAY PROCEDURES

### (For Crude Drugs)

**102. Type Process I.** Put into a 250 mil Erlenmeyer flask 15 gm. of the drug in fine powder with 150 mils of the appropriate immiscible solvent,\* Stopper the flask, shake well and let stand 10 minutes, then add 5 mils of water of ammonia and shake the mixture vigorously. Shake the flask continuously by aid of a mechanical shaker one hour or else shake well at frequent intervals during two hours. Add 15 mils of water (or enough to cause the powder to agglomerate) decant 100 mils of the clear fluid, guarding against evaporation. (It is well in warm weather to cool the flask to 15° C. or below before decanting, then stopper the measuring flask and bring its temperature to that at which the first measure ment was made and then adjust the measure accurately to 100 mils). Shake out the alkaloid with several successive portions of dilute sulphuric acid [See (23) and (27)] make the aqueous solution alkaline with ammonia and shake out once more with the appropriate immiscible solvent [See (26) and (28)]. Evaporate the solution by a gentle heat, redissolve the residue in a little alcohol or ether and evaporate, repeating the treatment once or twice. Dry the alkaloid to constant weight and weigh it, then as a rule, dissolve it in 5 mils (or sufficient) tenth-normal hydrochloric acid, and titrate excess of acid with volumetric alkali [See (70)].

**103. Type Process II.** Put into a 500 mils flask 15 gm. of the drug in fine powder, add 5 to 10 mils† of solution of lead subacetate previously mixed with distilled water sufficient to make 30 mils. Stopper the flask, shake and macerate at 50° C. 20 minutes, then add 270 mils of distilled water, shake, warm to 50° C. and macerate at that temperature 3 hours

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\*Most commonly a mixture of 1 volume of chloroform with five of ether, cooled to room temperature before measuring.

†The quantity to be judged by the amount of tannin and analogous compounds in the drug.



## TYPE ASSAY PROCESSES

with occasional shaking. Be sure that the temperature does not rise high enough to gelatinize starch. Cool the mixture, filter and to the filtrate add 0.75 gm. of dry sodium phosphate (or sufficient to precipitate the whole of the excess of lead). Filter once more, and from 100 mls of the filtrate, rendered alkaline (if necessary) with water of ammonia, shake out the alkaloid with the appropriate immiscible solvent (usually chloroform or a mixture of 3 volumes of chloroform with 2 of ether). Determine the alkaloid as in type process I. The method is suited particularly to the assay of drugs containing the water-soluble alkaloid colchicine. For drugs containing a large percentage of the less soluble alkaloids, use lead acetate in place of the subacetate.

104. **Type Process III.\*** Make a mixture of alcohol 3 mls, water of ammonia, 2 mls, ether 8 mls. With this, moisten 10 gm. of the finely powdered drug and pack in a miniature percolator, which may consist of the barrel of a small glass syringe. A little absorbent cotton is packed into the neck of the percolator and the drug is exhausted by slow percolation with ether or other appropriate solvent. One hundred mls of the solvent is generally quite sufficient, but make sure that exhaustion is complete by evaporating a ml of the percolate on a watch glass and adding a drop of decinormal sulphuric acid and a drop of Mayer's reagent. If a cloudiness is produced, continue the percolation until this test gives a negative result. Extract the alkaloid from the ethereal solution as in type process I. See (81).

105. **Type Process IV.** Follow type process I to the point (line 17) where the alkaloid has been brought into acid aqueous solution. Make up the solution to 100 mls, add strong hydrochloric acid 6 mls and 10 percent solution of silicotungstic acid sufficient to precipitate the alkaloid completely, collect the precipitate on a filter and wash it with water containing 1 per cent of hydrochloric acid, transfer it to a separator by aid of a little water, add 2 or 3 mls of water of ammonia and shake out carefully

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\*Method of Grandval and Lajoux., Pharm. Chem., 1893, 99, 152

with several successive portions of chloroform (20, 15, 15 and 10 mls, or sufficient) and proceed according to type process I (the last seven lines).

**106. Type Process V.** This is the same as type process I, except that the alkaloid is determined by direct instead of residual titration. The ethereal solution of the alkaloid is reduced by distillation or evaporation to a small volume, haematoxylin indicator is added, then in succession alcohol and water, and the solution is titrated with tenth-normal hydrochloric acid (73).

**107. Type Process VI.** Mix 5 gm. of the drug in fine powder with 1 gm. of calcined magnesia, and 5 mls of water, allow the mixture to become nearly dry and extract it in a soxhlet with chloroform or other appropriate volatile solvent. Determine the alkaloid in the resulting solution in the usual manner (14).

**108. Type Process VII.** The first steps of the assay are the same as in type Process I. The primary ethereal solution is evaporated to dryness, dissolved in 10 mls of 10 per cent sulphuric acid, and 50 mls of water, and 5 mls of Dragendorff's reagent (96) are added. The precipitate is collected and with the filter placed in a flask with 20 mls of 15 percent sodium hydroxide solution and 10 gm. of coarsely powdered crystallized sodium carbonate, and 50 mls of ether, and the mixture is shaken frequently during three hours. Into a stoppered flask are measured 100 mls of water, 20 mls of ether and 5 drops of iodeosin indicator, and any red color due to alkalinity of the glass is destroyed by careful addition of hundredth normal hydrochloric acid. Twenty-five mls of the ether solution are added, taking care to avoid loss of ether by evaporation, and the alkalinity of the mixture is determined by titration with hundredth-normal hydrochloric acid. (This method is preferable to an older one in which the alkaloids were precipitated from an acidulated solution with Mayer's reagent, the precipitate decomposed by digesting with sodium sulphite and the alkaloid extracted with ether after addition of an alkali.)

109. **Type Process VIII.** Extract the alkaloids from the drug by type Process I or otherwise. Weigh the alkaloidal residue and dissolve it in 5 mils (or a sufficient quantity) of decinormal hydrochloric acid. make up the volume of the solution to 25 mils. Use of this for the assay an aliquot containing 30 milligrams of the alkaloidal residue. Put into a 100 mil measuring flask 25 mils of tenth-normal iodine V. S. and 2 mils of 10 per cent hydrochloric acid. Add to this slowly with constant shaking the above aliquot, then fill the flask with water to the mark and shake vigorously until the alkaloidal periodide has completely separated, leaving the supernatant solution (which must be of a deep red color) perfectly transparent. Allow the solution to stand half an hour, then pipette off carefully 50 mils of it and titrate the excess of iodine with tenth-normal sodium thiosulphate V. S. Subtract the quantity in mils of thiosulphate used from 25 and multiply by the appropriate factor (84) to find the quantity of alkaloid in the aliquot taken.

109½. **Type Process IX.** Volatile alkaloids are most easily isolated by distilling with water and milk of magnesia (magma magnesiae, U. S. P.) or other suitable alkali. Otherwise the first step in the assay may be precipitation of the alkaloid from an aqueous solution with silico-tungstic acid. See (94) and (105), also (427).

## ROUTINE OF ASSAY PROCEDURES

### (For Fluidextracts)

110. **Type Process A.** Measure accurately with a pipette 5 mls of the fluidextract (if it contains less than 0.5 per cent of alkaloid, 10 mls; if it contains 1 percent, 4 mls; if 4 percent or more, 2 mls), transfer to a separator, add 25 mls of the appropriate immiscible solvent (in most cases a mixture of 3 volumes of chloroform with two of ether) then 1 mil of water of ammonia\* and 5 mls of water. Shake out warily for fear of emulsionizing. See (30) to (32). Repeat the shaking out with 20, 20 and 15 mls of the immiscible solvent (more if necessary for complete extraction of alkaloid). Unite the ethereal solutions, shake out with several successive portions (10 mls) of 2 percent sulphuric acid (22) and (27), and again extract the alkaloid by rendering the united aqueous solutions alkaline with ammonia and shaking out with the immiscible solvent. Evaporate the final alkaloidal solution by a gentle heat, redissolve the residue in a little alcohol or ether and evaporate, repeating this treatment once or twice, then dry the residue to constant weight and weigh, and determine the alkaloid by alkalimetric titration (70) or otherwise.

111. **Type Process B.** Mix 15 mls of the fluidextract with 150 mls of water, add slowly with constant shaking about 8 mls of solution of lead subacetate (just enough to give the mixture a distinctly sweet taste) and make up to 200 mls with water. Shake well at intervals during 20 minutes, filter and add to the filtrate 1 gm. of dry sodium phosphate (more if necessary to precipitate completely the

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\*In certain cases a different alkali is to be preferred, e. g., for cinchona in first extraction an alkali hydroxide or for physostigma an alkali bicarbonate. Before adding the ammonia it is generally best to add diluted alcohol to make 6 to 10 mls in all of hydroalcoholic fluid.

excess of lead), after 30 minutes filter once more (test first portion of filtrate with more sodium phosphate; if any turbidity is produced return the mixture and filtrate to its former container and add the necessary amount of sodium phosphate). Measure 133 mls of the filtrate (= 10 mls of the fluidextract), evaporate it to about 40 mls and shake out after addition of 1 ml of water of ammonia, with the appropriate immiscible solvent (25, 20, 20 and 15 mls; more if needed). Evaporate the solvent, redissolve the residue in 1 or 2 mls of alcohol, evaporate and dry the residue to constant weight and weigh. Determine alkaloid by alkalimetric titration (70) or otherwise.

**112. Type Process C.** F. A. Thompson's sawdust process.\* Place in a capsule 5 to 7 gm. of purified sawdust (U. S. P. IX, p. 546) pour gradually onto this 10 mls of the fluidextract. (If the fluidextract contains more than 1 percent of alkaloid, dilute a portion of it with 50% alcohol to reduce to approximately that strength, and use for the assay 10 mls of the dilution, noting of course exactly how much of the original fluidextract this represents.) Mix thoroughly with the sawdust, which must be sufficient in quantity to absorb the whole of the fluid, dry at a temperature not exceeding 50° C., transfer to a suitable flask, cleaning the capsule if necessary with a little sawdust moistened with diluted alcohol. Proceed then as in the assay of a crude drug by type process I (102). (This process is resorted to in case of fluidextracts which are liable to form obstinate emulsions. It is official in U. S. P. IX for the fluidextracts of aconite, cinchona, ipecac and pilocarpus.)

**113. Type Process D.** This is a variant of type process C, substituting for the sawdust 0.5 to 1.0 square meter of clean cheese cloth, which has the advantage over purified sawdust that it is easily procurable. The cheese cloth (or absorbent surgical gauze) is placed in a flat-bottomed dish and the fluidextract dropped upon it carefully. Drying of the gauze can be hastened by hanging it up in a warm place. It is not necessary or desirable that it

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\*Proc. Mich. Pharm. Assoc., 1891.

should be completely dried, the object of the drying being merely to get rid of alcohol. Any portion of the fluidextract that may have remained in the dish can be easily wiped out with a bit of the gauze. Other absorbents which may be employed in place of sawdust are other vegetable fibers that are not liable to soften and mat together, e. g. cottonwood fiber or coarse twine cut in short pieces. Pumice in fragments as large as a hemp seed answers a good purpose.

**114. Type Process E.** Applicable where there is likelihood of emulsification. Put into a separator 10 mils of the fluidextract (5 mils if it contains as much as 2 per cent of alkaloid), add 80 mils of ether and 2 or 3 mils of water of ammonia and shake gently but continuously 2 or 3 minutes and when separation has taken place transfer the ether to a suitable flask or beaker; shake out the residual alkaline solution with two successive portions of ether (10 mils) exercising due caution to avoid emulsification. Evaporate the combined ethereal solutions nearly to dryness by a gentle heat. The alkaloidal residue is often pure enough for titration. If not, dissolve it in a little diluted sulphuric acid (filter if necessary) then wash the acid solution twice with chloroform (10 mils), shake out the alkaloid with ammonia and ether or other appropriate immiscible solvent and determine in the usual manner.

**115.** An expedient suggested by M. H. Webster\* for avoiding emulsification in dealing with fluidextracts, consists in adding to the fluidextract five times its volume of absolute alcohol containing in solution 1 per cent of tartaric acid. This precipitates along with albuminous and gummy matter, nearly all the ammonia that may be present. The solution is filtered and the precipitate washed with absolute alcohol; the filtrate and washings are evaporated to a solid extract, which is dissolved in water acidified with tartaric acid, leaving behind resins, chlorophyll and fatty matter. From the aqueous solution the alkaloids are generally easily extracted by the usual

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\*Am. Journ. Pharm., July 1907, 301-7.

shaking out process, with no danger of emulsification.

**116. Type Process F.** Put into a suitable flask 1 gm. of freshly slaked lime, add 10 mls of the fluid-extract, previously mixed with 80 mls of alcohol;\* shake well at frequent intervals during 20 minutes, decant through purified cotton into a 100 mil measuring flask; wash the residue with several portions of alcohol to make up 100 mls. Filter, acidify faintly an aliquot part with sulphuric (or tartaric) acid, and evaporate off alcohol at a gentle heat. Dissolve the residue in 10 mls of water containing a little sulphuric acid, filter, wash residue with water, transfer filtrate and washings to a separator, shake out with 2 or more portions of ether (15-20 mls) to remove fatty and waxy matter, render alkaline with ammonia and shake out the alkaloid with the appropriate immiscible solvent.

**117. Type Process G.** Put into a 100 mil measuring flask 10 mls of the fluidextract (5 mls if the fluid contains 2 per cent or more of alkaloid), add 6 mls of strong hydrochloric acid and water sufficient to make 100 mls. Filter with aid of powdered pumice if necessary. To an aliquot portion of the filtrate add silicotungstic acid sufficient to precipitate the whole of the alkaloid, collect the precipitate on a filter and complete the assay as in (94).

**118. Type Process H. (Precipitation by Picrolonic acid).** Extract the alkaloids from 10 mls of the fluidextract as in type process A. Evaporate the original solution in ether-chloroform to half its volume, add 5 to 10 mls of a tenth-normal alcoholic solution of picrolonic acid and let stand 24 hours for crystallization of the alkaloid-picrolonate. Collect this on a filter, wash slightly with a mixture of ether and alcohol and dissolve it in warm dilute sulphuric

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\*If a heavy precipitate of gummy matter is thrown down, allow this to subside, decant the solution which will generally still be turbid, upon the slaked lime, with which it is to be shaken well. Meanwhile dissolve the precipitate in about 6 mls of diluted alcohol, add 0.25 gm. slaked lime, mix well and add the mixture to the alcoholic solution, shake well together and proceed as in the text.

acid. Shake out the liberated picrolonic acid with acetic ether, leaving the alkaloid in aqueous solution in the form of sulphate. Finally render the solution alkaline and shake out the alkaloid with the appropriate immiscible solvent.

119. **Type Process I.** Extract the alkaloid in crude form from 10 mls of the fluidextract as in type process A or B. Dissolve this in weak sulphuric acid and add water to make up to 20 mls. Precipitate the solution with a moderate excess of potassium-bismuth iodide solution. After 15 minutes collect the precipitate on a filter and wash twice with diluted sulphuric acid (5 mls). Proceed in the manner described in (97), beginning with line 9.

120. **Type Process J.** Introduce 5 or 10 mls of the fluidextract into a perforator (56), render acid and wash thoroughly with petroleum benzin, then make alkaline and extract the alkaloid with the appropriate immiscible solvent.

121. **Type Process K.** Evaporate off alcohol at a low temperature, add plaster of Paris mixed with a little sodium bicarbonate, allow the mixture to set and harden. When sufficiently dry, pulverize and extract the alkaloid in a soxhlet, or else simply by slow percolation, with an appropriate solvent. As a rule it will be necessary to dissolve the crude alkaloid in weak sulphuric acid, filter if necessary, wash the acid solution with ether to remove impurities, render the solution alkaline, and shake out the alkaloid with the appropriate immiscible solvent.

#### ASSAY OF EXTRACTS

122. **Solid extracts** are to be converted into fluid-extracts by dissolving in diluted alcohol when that is practicable. In many cases they cannot be dissolved as a whole, yet when treated simultaneously with diluted alcohol (or even with water), water of ammonia and chloroform, they dissolve so completely that the alkaloid may be extracted in crude form by shaking out with chloroform. If the diluted alcohol yields a turbid mixture having only suspended particles of



undissolved matter, we may use sawdust or cheese cloth as an absorbent, dry and treat as crude drug. See (112) and (113).

**123. If the extract is an aqueous one,** heavily loaded with gummy matter, we may exhaust it with alcohol by the following treatment: Dissolve the extract (2 gm.) as completely as possible in a mixture of alcohol and water in such proportion as experiment shows to have the greatest solvent action. Add to the turbid solution three to six times its volume of strong alcohol, causing precipitation of the gummy substances. Decant the clear alcoholic solution into a wide beaker, redissolve the gum in 5 mls of water, add alcohol drop by drop with stirring, to the point of incipient precipitation, then add at once 10 mls of strong alcohol to reprecipitate the gum. Decant the solution into the beaker and repeat the treatment with alcohol once more. As a rule no alkaloid will remain in the gummy matter after this treatment, but this should be made certain by test with Mayer's or Wagner's reagent. The united alcoholic solutions are to be concentrated by evaporation, sulphuric acid is to be added and the solution is to be diluted and filtered and the alkaloids extracted in the manner which seems most feasible. If the gummy residue shows presence of alkaloid it may be dissolved in water and assayed according to (116).

**124. In the case of alcoholic extracts** heavily loaded with chlorophyll, fats or resinous or waxy matter, the following procedure is a good one. Soften the extract by warming in a capsule with a little diluted alcohol. Add to the syrupy extract 0.5 ml of diluted sulphuric acid (10%) and treat the mixture with successive portions (5-10 mls) of ether, decanting these into a separator until the most of the chlorophyll, etc., has been taken up. Put into the separator 5 mls of water with 5 drops of diluted sulphuric acid. Shake judiciously, let separate and draw off the acid fluid into the capsule containing the residue of the extract, which will be now mostly soluble in water. Dissolve in 10 mls (altogether) of water, transfer to a separator, rinse the capsule with several small por-

tions of water and of ether, shake out twice with ether to remove the last traces of chlorophyll, etc., then make alkaline and shake out with the appropriate immiscible solvent.

#### GENERAL PROCESSES FOR THE ASSAY OF EXTRACTS.

**125. Processes identical in principle** with those underlying the various type processes for fluidextracts may be easily deduced from the text describing those processes. See (102) to (121). In the assay of **powdered extracts**, it is to be remembered that these often contain insoluble constituents such as calcined magnesia, powdered drug, starch, etc., so that a general routine process cannot be laid down. One must depend on application of familiar general principles.

**126.** Alkaloids may often be separated from **syrups** by the following method, suggested by E. Kohn-Abrest:\* Treat the syrup with 4 times its volume of alcohol (in case of syrups having a specific gravity below 1.14, absolute alcohol should be used) and about its own weight of dry potassium carbonate. The sugar is precipitated as a pasty mass, the alkaloids going into solution in the alcohol, which is readily poured off. The residue may be washed with additional alcohol to extract residual alkaloid. The solvent is distilled off, and the residue treated with absolute alcohol or with a mixture of alcohol and chloroform to obtain the alkaloid in approximately pure form, or the residue may be treated with acid to bring the alkaloid into aqueous solution, the final extraction being effected according to general principles.

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\*Bull. Soc Chim., 1912 (IV), 11, 73-5.

## VOLATILE OILS

127. An interesting, but for the analyst a difficult, group of products are the essential oils distilled from various plants. A few of these, e. g. oil of gaultheria, consist almost wholly of a single chemical compound, but as a rule the oil is of complex composition. Hence unusual importance attaches to the physical properties of the product, particularly to specific gravity, specific refraction, behavior towards polarized light, boiling point (without or with fractional distillation), congealing point, and of course odor and flavor. Solubility in alcohol of 50, 70 or 90 percent strength is a property of practical importance, particularly as a criterion of freedom from adulterations.

128. **Hydrocarbon compounds** are present in most of the essential oils, and are the most abundant if not the only constituents in many instances, but it is not to these that the oils owe the distinctive odor or flavor for which they are particularly valued. In a preliminary examination of an essential oil, a **low specific gravity** indicates a preponderance of hydrocarbons. Quantitative determinations of these constituents, however, are not often attempted, inasmuch as in most cases they have no relation but a negative one to the value of the oil.

129. **It is the oxygenated compounds** which in most cases are of importance in the standardization of essential oils. These include alcohols, aldehydes, esters in great numbers, ketones and phenols, each class having its own general methods of assay. The esters especially are of interest and it is these which are most readily split into their component parts. They are generally acted on by caustic alkalies, which combine with the acid constituent by a reaction similar to that in which a fixed oil is made to yield soap, so that the process is known as saponification. Thus methyl salicylate which occurs nearly pure in essential oil of gaultheria, when treated with potassium

hydroxide solution yields potassium salicylate and methyl alcohol, becoming thus completely soluble in water. The reaction is quantitative, and it is easy to decompose the potassium salicylate in turn and weigh or titrate the salicylic acid formed. From this the quantity of methyl salicylate is easily deduced. For saponification of esters, a volumetric alcoholic solution of sodium or potassium hydroxide is generally used (half-normal alcoholic potassium hydroxide V. S. of U. S. P.). In case the oil is neutral in reaction, the quantity of saponifiable ester it contains is ascertained by titrating the excess of alkali and calculating from the quantity of alkali neutralized in the saponification, the quantity of ester present in the oil.

130. **The saponification procedure** is carried out in the following routine manner; two mls of the oil are introduced into a tared flask and accurately weighed, 10 mls of the half normal alcoholic potassium hydroxide solution (in special cases more may be required) are added and the mixture is heated on a water bath under a reflux condenser one hour. The cooled solution is titrated with half normal sulphuric acid, using phenolphthalein as indicator; the quantity of acid consumed is subtracted from that of the half normal alkali and the difference is multiplied by the appropriate factor to find the quantity of ester in the sample examined. A number of these factors will be found on page 568 of U. S. P. IX.

131. **The method is applicable to some alcohols** (e. g. bórneol, menthol, santalol) as well as to esters. It is not applicable in the case of aldehydes, which indeed if present vitiate the results of determinations of esters or alcohols on account of progressive decomposition of aldehydes by alkalies.

132. **Assay by acetylizati<sup>o</sup>n.** Certain alcohols (e. g. borneol, geraniol and menthol) react with acetic anhydride with quantitative formation of the corresponding esters. The process is conducted in the following routine manner: Place in a small flask fitted by a ground glass joint with a long tube to serve as a reflux condenser (acetylizati<sup>o</sup>n flask) 10 to 20 mls of the oil with an equal volume of acetic anhydride

and 1 to 2 gm. of anhydrous sodium acetate, and boil gently 1 to 2 hours. When cool, add 50 mls of water and heat the mixture 20 to 30 minutes on a water bath to convert the excess of acetic anhydride to acetic acid. Separate the oily layer and wash it carefully in succession with solution of sodium carbonate and with water until neutral to litmus. Treat the oil with anhydrous sodium sulphate or with fused calcium chloride to remove moisture, saponify the dried oil (130) and so calculate the quantity of the alcohol originally present.

**133. An alternative method of acetylation** has been proposed by A. Verley and Fr. Bölsing,\* pyridine being used in place of sodium acetate. It is claimed that esterification is more rapid and complete than under the customary procedure. A mixture of acetic anhydride 120 parts and pyridine 880 parts is employed. This is titrated with standard alkali before and after heating with the alcohol or phenol to be determined. The method has been applied successfully in determinations of ethylic, amyllic and cinnamic alcohols, menthol, phenylglycol, glycerol, phenol, betanaphthol, guaiacol, thymol, eugenol, carvacrol and santalol, but not of geraniol, terpineol, linalool, vanillin or benzyl alcohol.

**134.** For details of the method of determining in an oil the proportion of an alcohol present in the form of esters and the total percent of the same alcohol, see under Oil of Peppermint (568) to (571).

**135. Phenols** may often be determined with approximate accuracy by merely shaking the oil with a five percent solution of sodium hydroxide, which will form with the phenol a water-soluble compound. The test may be made in a cassia flask,† twelve hours being allowed for complete separation of the residual oil. The diminution in volume of the oil is taken as

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\*Bericht, 1901, 3354-8.

†A measuring flask with slender neck, holding to the mark, near the base of the neck, 100 mls, the portion of the neck immediately above the mark (10 mls) graduated to tenth- or twentieth-mils.

an approximate measure of the phenol present. Separation of the oil may be hastened by the addition of a definite volume of petroleum benzin, this volume being deducted from that of the residual oil. A more exact determination of phenols may be made by the method of Schryner\* in which sodamide is made to react with the phenol, which must be strictly anhydrous, with consequent formation of a definite quantity of ammonia.

### 136. Determination of Aldehydes and Ketones

The method of determining aldehydes which is best known is that described under oil of cinnamon (542), in which the oil is treated with sodium bisulphite, the aldehydes being converted thus into water-soluble compounds, and the diminution in volume of the oil indicating the quantity of aldehyde present.

**137. Improved Sulphite assay process.** In this a neutral sodium sulphite replaces the bisulphite of the foregoing test. It yields results differing somewhat from those where bisulphite is used, but believed to be more accurate. It is adopted in the U. S. P. IX for determination of carvone in oil of caraway and in oil of spearmint, and is given in detail in (536). Good results are reported from the use of this method in the assay of oils of bitter almond, caraway, cassia, cinnamon, citronella, cumin, lemon grass and pennyroyal. In the cases of cumin and pennyroyal, litmus is to be preferred to phenolphthalein as indicator of neutrality.

**138. The Hydroxylamine method.** Many aldehydes and ketones, when acted upon by hydroxylamine in alcoholic solution, form oximes quantitatively. By determining the residual hydroxylamine, the quantity of the aldehyde or ketone is arrived at. The method is used in determination of citral in oil of lemon (676).

**139. The Semicarbazide method.** The reagent, otherwise described as carbamic hydrazide, converts aldehydes and ketones into crystallizable compounds called semicarbazones. To prepare the reagent† dissolve 130 parts of hydrazine sulphate,

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\*Journ. Soc. Chem. Ind., 1899, 18, No. 6.

†Thiele and Stange, in *Annalen*, 1894, 281, 19.

55 of sodium carbonate and 85 of sodium cyanide in 1000 parts of water. Add one and one half volumes of alcohol, let stand a few hours and filter. To 250 mls of the reagent, add 10 gm. of the sample, shake together and let stand 24 hours. Collect the crystals on a tared filter, add water to the filtrate to precipitate residual semicarbazone, which may be crystallized from alcohol and added to the first crop of crystals.

**140. General method for determining essential oils** in spices and drugs. The sample (20 gm.) is extracted in a suitable extraction apparatus by steam distillation, an operation requiring  $1\frac{1}{2}$  to  $2\frac{1}{2}$  hours. To the distillate, amounting to 400 to 900 mls, is added one fourth its weight of sodium chloride (freed from impurities insoluble in water). When this has been dissolved, the solution is transferred to a flask which it fills to the neck, 50 mls of light petroleum (boiling at  $20^{\circ}$  C. to  $35^{\circ}$  C.) are added and the fluids are shaken together for 30 minutes and then set aside 2 hours for complete separation. The neck of the flask, which should be narrow, has two marks including a volume of exactly 50 mls. A clear 25 percent solution of salt is added cautiously to bring the lower level of the petroleum solution to the lower mark, and fresh petroleum is added to bring the upper level accurately to the upper mark. One half the petroleum solution (thoroughly mixed) is removed to a flask fitted with a tube by which dry air is driven through it in a gentle current, and another delivering the air and vapor of petroleum through a fine platinum jet against the tip of a small Bunsen flame. When the vapor no longer produces a luminous cone, the air current is shut off, and the weight of the essential oil which remains in the tared portion of the apparatus is weighed. Some loss of the more volatile constituents of the oil will always take place, but rarely enough to vitiate the result seriously.

**141. Determination of amount of a volatile oil in a spirit** or alcoholic solution (e. g. in a flavoring extract). The following is the Howard-Mitchell

method,\* adopted provisionally by the A. O. A. C. for flavoring extracts of peppermint and spearmint. Pipette 10 mls of the extract into a Babcock milk bottle, add 1 ml of carbon disulphide, mix thoroughly, then add 25 mls of cold water and 1 ml of strong hydrochloric acid. Close the mouth of the bottle with the thumb and shake vigorously, whirl in a centrifuge 6 minutes and remove all but 3 or 4 mls of the supernatant liquid, which should be practically clear, by means of a glass tube of small bore, with aspiration. The carbon disulphide is then removed by means of a filter pump, immersing the bottle in water, first at 70° C. (3 minutes), finally at 100° C. (45 seconds). The bottle meanwhile is shaken vigorously every 15 seconds, and the shaking is continued after removing it from the water bath until it is cool, suction being maintained in the meantime. Finally the bottle is filled at room temperature with saturated salt solution, centrifuged two minutes and a reading is taken of the volume of the separated oil from the top of the meniscus. In the case of oil of wintergreen on account of its high specific gravity use in place of the salt solution a mixture of 1 volume of concentrated sulphuric acid with 3 volumes of a saturated solution of sodium sulphate.

**142. Method of Hartvet and West,\*\*** adopted provisionally by the A. O. A. C. for flavoring extracts of anise and nutmeg. Place in a Babcock milk bottle 10 mls of the sample, add 1 ml of hydrochloric acid sp. gr. 1.08, then sufficient of a 25 percent salt solution, previously heated to 60° C., to fill the bottle nearly to the neck. Stopper the bottle and let it stand in a water bath at 60° C. 15 minutes, occasionally giving it a twisting motion, then centrifuge 10 minutes at about 800 revolutions per minute. Add salt solution until the separated oil rises into the neck of the bottle and centrifuge again 10 minutes. If the separation is not satisfactory, or the liquid is not clear, cool to about 10° C. and centrifuge for an additional

\*For other methods of C. D. Howard see Journ. Am. Chem. Soc., 1908, 608-11, and Journ. Ind. and Eng. Chem., 1911, 252.

\*\*Journ. Ind. and Eng. Chem., 1909, 84.



10 minutes. Read off the volume of the separated oil and multiply by 2 to find the volume percent present in the sample.

### FIXED OILS AND FATS

143. Standards for fixed oils and fats based on chemical assays are practically out of the question, since the several glycerides entering into their composition are difficult of quantitative separation. Differences in specific gravity of the oils are so slight that this physical character has little importance. With few exceptions these compounds have no effect on polarized light. The melting or congealing point of the several fats and oils is generally a distinctive character of importance, although this alone is not an effective safeguard against sophistication. There are however two characters which are of especial importance in discriminating these products, viz. the saponification and iodine absorption values.

144. **Saponification Value.** This is the quantity of potassium hydroxide consumed in saponifying 1000 parts of the fat or oil. The oil or melted fat is filtered, two mls of it are transferred to a tared 250 ml flask and weighed accurately, and 25 mls of half-normal alcoholic potassium hydroxide are added. The flask is fitted with a condensing tube (about 75 cm. in length and 7.5 mm. in diameter), and heated on a water bath half an hour, with occasional gentle shaking or rotating. Titrate the excess of alkali with half-normal hydrochloric acid, using phenolphthalein as indicator. Make a blank test, omitting the oil. The difference (in mls) in the two titrations multiplied by 28.055 and the product divided by the weight of the sample taken gives the saponification value of the oil or fat.

145. **Iodine absorption value.** This is the quantity of iodine absorbed by 1000 parts of the oil or fat under the conditions of the test. An accurately weighed sample of the oil or fat\* is dissolved in 10

\*About 0.8 gm. of a solid fat or 0.3 gm. of an oil (for cod liver oil 0.18 to 0.2 gm.; for linseed oil, 0.15 to 0.18 gm.).

mils of chloroform in a 250 mil glass-stoppered flask. Add 25 mils of the official iodobromide test solution.\*\* Stopper the flask and set aside half an hour (in case of castor oil or linseed oil, one hour) in a dark place. If at the end of this time the color of the mixture is not still brown, a new test must be started, using a smaller quantity of the oil or fat. To the brown mixture add in succession 30 mils of a solution of potassium iodide (20 gm. to 100 mils), 100 mils of distilled water and, from a burette, little by little, with constant vigorous shaking, tenth-normal sodium thiosulphate until the color of the mixture is nearly discharged. Add now a few drops of starch solution and continue the titration with sodium thiosulphate to complete disappearance of blue color. Carry out meanwhile a blank test, omitting the oil or fat. Multiply the difference in mils in the result of the two titrations by 1.269 and divide the product by the weight of the sample taken to find the iodine value of the oil or fat.

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\*\*Dissolve in 1000 mils of glacial acetic acid 13.2 gm. of powdered iodine, by aid of a gentle heat. Titrate 20 mils of the cooled solution with tenth-normal sodium thiosulphate to determine the quantity of iodine present. Add to the balance of the solution for each gm. of iodine it contains 0.62969 gm. of bromine (the approximate amount being usually 3 mils).

**Part II.**  
**Detail of Methods of Assay**  
**and Standardization of**  
**Individual Drugs**

## Chapter I—Solvents

### ALCOHOL

146. **Alcohol in simple aqueous solution** is determined customarily by the specific gravity of the mixture, which may be taken either with a common hydrometer having an open scale, by a specially graduated instrument (alcoholometer) which gives by direct reading the volume percent of alcohol, or by use of a Westphal balance or of a pycnometer (specific gravity bottle). The last gives the most exact results, being used in connection with tables which give the alcoholic strength corresponding with any specific gravity to the hundredth of an integral percent. See U. S. P. IX, pp. 633-637.

147. **Preparations containing other substances** besides alcohol and water must be distilled so as to obtain a mixture of water and alcohol only. If there are other volatile substances present, these must either be held back in the still or else they must be separated from the distillate by an appropriate solvent as hereafter explained. For routine work, a small copper still with capacity of 300 to 500 mls, provided with a block tin condensing "worm" is most convenient. Otherwise a glass flask with suitable condenser may be employed, best one provided with some device, such as a bulbed neck (Ladenburg's distilling flask), to prevent frothing over of the fluid in the still.

148. Use for the distillation 25 mls of the sample, **which for accurate work must be measured at 60° F. (15.56° C.).** Add 50 mls of water and distil slowly (not more than a mil in 30 seconds at the start) using as a receiver a 50 mil measuring flask, containing 5 to 10 mls of distilled water and so arranged that the delivery tube discharges below the surface of the water. (After 10 to 20 mls of distillate, according to strength of sample, have come over, the receiver should be lowered so that the delivery tube no longer dips into the distillate.) It is well to use for the distillation, where the sample contains less than 15

percent of alcohol, 50 mls of the sample with 25 mls of water added; where the strength is above 50 percent, 12.5 mls is a sufficient quantity of the sample, with about 50 mls of water, the measurement of the sample being made always at 15.56° C. When the receiver is filled nearly to the mark, discontinue the distillation,, bring the temperature of the distillate to 15.56° C. and add enough distilled water at or slightly below the same temperature to bring the volume to exactly 50 mls. Allow this to stand half an hour in the weighing room, or else bring its temperature approximately to that of the air in the weighing room, note the exact temperature of the distillate and take its specific gravity by pycnometer or Westphal balance,\* ascertaining then from the alcohol tables of the Pharmacopoeia, or the condensed table on pp. 72-73, the percent by volume of alcohol present. Multiply this by 2 if 25 mls of the sample were used, by 4 if 12.5 mls were used, to find the alcoholic strength of the sample.

**149. Thus far, it is assumed** that no volatile substances besides alcohol and water are present in the sample. In fact, this is rarely the case where the sample examined is a medicinal preparation. The very small quantity of volatile oils, representing the flavoring constituents of the medicine, may generally be disregarded; usually they do not even render the distillate turbid. It is otherwise where a volatile oil is present in such quantity as to cause decided turbidity in the distillate—still more where the oil separates in visible drops. In such cases, if exact results are sought, it is necessary to treat the distillate with a solvent which takes up the volatile oil without removing with it a part of the alcohol. Petroleum benzin (gasoline) is well suited for this use. Transfer the distillate to a separator together with rinsings from the receiver, add common salt (or calcium chloride) enough to nearly saturate the liquid and shake out with two or three successive portions (15 mls) of

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\*The Westphal balance can be used at temperatures other than 15. 56° C. only if the plummet is made of glass.

petroleum benzin. Draw off the salt solution into a distilling flask (or metallic still), wash the benzin with 25 mls of a strong solution of sodium or calcium chloride, which is used also to rinse the separator previously used, and is then transferred to the still. By a second distillation, conducted in the same manner as the first, a product is obtained from the specific gravity of which the alcoholic content of the sample is deduced as already explained. The same method may be used to separate ether and many similar volatile fluids from alcohol.

149½. Presence in the distillate of methyl alcohol is a possibility not to be overlooked. Tests for this impurity will be found in (173) to (177). The simplest means of detecting it is by the Zeiss immersion refractometer, which enables us also to determine approximately the amount present. See (180) to (182).

150. Should the original distillate show by its odor presence of **camphor**, **chloroform**, or any similar organic compound, the same method of purification must be carried out. If the distillate shows a strong acid or alkaline reaction, indicating presence of a notable quantity of a volatile acid or base, a second distillation is necessary, with addition of milk of magnesia or some other alkali, if the reaction is acid, or of diluted sulphuric acid if it is alkaline. If the presence of a volatile acid or base in the sample is known in the first instance, these will of course be neutralized before distillation. The case may occur where there is present both a volatile base and a volatile acid, when it becomes necessary to make two distillations, the first to eliminate the alkali, the second the acid.

151. **Annoyance is often caused** in distilling tinctures, etc., by excessive frothing. The cause may be assumed to be usually the presence of a saponin. In the majority of cases, addition of some diluted sulphuric acid obviates the trouble, especially if heat is applied at first quite gradually. Addition of a little

paraffin is another remedy. The Ladenburg still, or some equivalent device may be employed with satisfactory results in such cases.

152. The directions commonly given with regard to measuring sample and distillate are simply to take care that these two measurements be made at the same temperature. This is correct if the distillate has the same volume as the original sample, not otherwise.\* It must be remembered that volume percentages for alcohol assume that the volumes are measured at 15.56° C. But the co-efficient of expansion of alcohol is much greater than that of water. Therefore it is necessary to make measurements of sample and distillate, when these are not of the same volume, at the standard temperature for alcohol, viz. 15.56° C.

153. Under the Food and Drugs Act, the quite unreasonable requirement is made that all medicinal preparations containing alcohol shall bear on their labels the percent of that constituent present. The intention was to indicate thus the potency of the preparation as an intoxicant. Such a requirement in case of patent or proprietary medicines would serve in a measure to safeguard the public against the danger of alcoholic addiction from the use of what were offered as medicines. But when the requirement is made to apply to fluidextracts and tinctures of which a few minims, or even a few fluidrachms, constitute a lethal dose, it imposes on the pharmacist a burden wholly without justification. In the case of **fluid-extracts** the requirement is particularly inept, since the alcohol content of these necessarily varies very widely owing to the large and variable proportion of extractive they carry, and to equally great variations in the quantity of water the drug contains. The practical consequence is that the figures which the law requires to appear on the labels can be considered only an average from which variations of ten percent above or below must be considered not excessive.

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\*At least when more than 15 or 20 percent of alcohol is present.

# CONDENSED ALCOHOL TABLE

sufficiently exact for the ordinary requirements of the pharmacist.

<i>Percent Alcohol Volume</i>	<i>Specific Gravity Approximate at 25° C. Baumé 15.560</i>	<i>Fractional Percent Equib. to Difference of 0.0001</i>	<i>Temperature Correction for 1° C. †</i>	<i>Temperature Correction for 1° F. ‡</i>
0	0.99825	0.0667	0.155	0.086
1	0.99675	0.0680	0.160	0.089
2	0.99528	0.0689	0.164	0.091
3	0.99383	0.0704	0.168	0.093
4	0.99241	0.0714	0.173	0.095
5	0.99101	0.0735	0.179	0.099
6	0.98965	0.0757	0.185	0.102
7	0.98833	0.0775	0.193	0.107
8	0.98704	0.0794	0.201	0.112
9	0.98578	0.0800	0.208	0.116
10	0.98453	0.0806	0.216	0.120
11	0.98329	0.0813	0.224	0.124
12	0.98206	0.0826	0.232	0.129
13	0.98085	0.0826	0.242	0.134
14	0.97964	0.0840	0.251	0.139
15	0.97845	0.0847	0.263	0.146
16	0.97727	0.0862	0.278	0.154
17	0.97611	0.0885	0.294	0.163
18	0.97498	0.0885	0.311	0.172
19	0.97385	0.0893	0.325	0.181
20	0.97273	0.0877	0.336	0.187
21	0.97159	0.0862	0.346	0.192
22	0.97043	0.0855	0.355	0.197
23	0.96926	0.0833	0.364	0.202
24	0.96806	0.0826	0.371	0.206
25	0.96685	0.0813	0.378	0.211
26	0.96562	0.0806	0.386	0.214
27	0.96438	0.0793	0.391	0.217
28	0.96312	0.0781	0.389	0.221
29	0.96184	0.0761	0.402	0.223
30	0.96054	0.0752	0.406	0.225



<i>Percent Alcohol Volume</i>	<i>Specific Gravity Approximate at 25° C. Basis 15.56</i>	<i>Fractional Percent Equivalent to Difference of 0.0001</i>	<i>Temperature Corrections for 1° C. †</i>	<i>Temperature Corrections for 1° F. ‡</i>
31	0.95921	0.0735	0.407	0.226
32	0.95785	0.0719	0.408	0.227
33	0.95648	0.0694	0.407	0.226
34	0.95500	0.0680	0.406	0.225
35	0.95353	0.0658	0.404	0.224
36	0.95201	0.0649	0.402	0.223
37	0.95047	0.0633	0.401	0.222
38	0.94889	0.0621	0.399	0.222
39	0.94728	0.0606	0.398	0.221
40	0.94563	0.0588	0.396	0.221
41	0.94393	0.0578	0.395	0.219
42	0.94220	0.0571	0.392	0.218
43	0.94045	0.0562	0.389	0.217
44	0.93867	0.0553	0.387	0.215
45	0.93686	0.0540	0.384	0.214
46	0.93501	0.0529	0.382	0.212
47	0.93312	0.0521	0.379	0.211
48	0.93120	0.0513	0.377	0.209
49	0.92925	0.0505	0.375	0.208
50	0.92727	0.0500	0.373	0.207

\*Apparent specific gravity by pycnometer which holds 15.56° C. (60° F.) exactly 100, 50 or 25 gm.

†Additive if temperature is below, subtractive if above 25° C.

‡Additive if temperature is below, subtractive if above 77° F.

Example. A distillate measuring 50 mls, from 25 mls of a given sample, shows apparent specific gravity at 20° C. (68° F.) = 0.98501. Find in Column II the nearest higher figure, which is 0.98578, corresponding with 9 percent (vol.) of alcohol. Difference (0.98578 — 0.98501) = 0.00077. Fractional percent then =  $7.7 \times 0.0800$  (from column III) = 0.616, making the percent (uncorrected) 9.616. Correction for temperature (25° — 20° = 5°) =  $5 \times 0.208$  = +1.04, making corrected percent of distillate 10.656. Since distillate has twice the volume of the sample, multiply this figure by 2, making the percent of alcohol in the sample 21.31.

In absence of a centigrade thermometer, the correction for temperature can be deduced from column V.

## GLYCERIN

154. For **glycerin answering the U.S.P. requirements** the specific gravity may be assumed to indicate with practical accuracy the percent of glycerol present. The index of refraction may be used also. The following table is believed to be sufficiently correct for ordinary purposes. Its figures are substantially those of Skalweit as quoted in Allen's Commercial Organic Analysis, third edition.

Percent Glycerol	Specific Gravity at 15° C.	Index of Refraction (sodium ray at 15° C.)
0	1.0000	1.3330
5	1.0118	1.3390
10	1.0239	1.3452
15	1.0363	1.3516
20	1.0490	1.3581
25	1.0620	1.3647
30	1.0751	1.3715
35	1.0884	1.3785
40	1.1018	1.3854
45	1.1153	1.3924
50	1.1290	1.3996
55	1.1428	1.4069
60	1.1567	1.4144
65	1.1707	1.4220
70	1.1848	1.4295
75	1.1989	1.4369
76	1.2017	1.4384
78	1.2073	1.4414
80	1.2129	1.4444
82	1.2184	1.4475
84	1.2238	1.4505
86	1.2292	1.4535
88	1.2346	1.4565
90	1.2399	1.4595
92	1.2450	1.4625
94	1.2500	1.4655
96	1.2551	1.4684
98	1.2601	1.4712
100	1.2652	1.4742

155. **Among chemical determinations** of glycerol  $[C_3H_5(OH)_3]$  in glycerin of such purity that it conforms to U. S. P. requirements, the modified

dichromate method of F. T. Bradt\* answers sufficiently well the requirements of the pharmacist. Introduce into a tared, stoppered weighing bottle 4 mls of the glycerin if of approximately official strength—otherwise a quantity which is estimated to contain about 4.5 gm. of glycerol. Weigh accurately, then dilute this to exactly 100 mls and take for the assay exactly 5 mls of the dilution. Add 50 mls of tenth-normal potassium dichromate solution and 25 mls of strong sulphuric acid and heat on a steam bath 20 minutes. Cool, add one gramme of potassium iodide (free from iodate) and after 10 minutes dilute to 150 mls with water. Titrate the liberated iodine with tenth-normal sodium thiosulphate using starch as indicator. Subtract the quantity in mls of the thiosulphate solution from 50 and multiply the remainder by 0.0006576 gm. to find the quantity in grammes of glycerol contained in the sample taken for assay.

156. Oxidizable impurities in the sample will make the result of the assay high. If the presence of alcohol is suspected, add an equal volume of water and evaporate at a temperature below 100° C. before adding the dichromate solution.

157. **Hehner's Dichromate method,**† modified\*\*, for determination of glycerol in commercial glycerin. The method is the same in principle as that of Bradt, but more circumstantial and better suited for testing glycerin of inferior quality. The reagents required are (1) pure potassium dichromate, powdered and dried at 110° to 120° C., in air free from dust or organic vapors; (2) dilute dichromate solution, made by dissolving 7.4564 gm. of the pure potassium dichromate in sufficient distilled water to make one liter at standard temperature; (3) ferrous ammonium sulphate whose exact deoxidizing power is to be determined by titration with the foregoing solution; (4) freshly pre-

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\*Journ. Am. Pharm. Assoc., Jan. 1915, 78-81.

†Journ. Soc. Chem. Ind., 1889, 4.

\*\*From Report of a Committee of Industrial Chemists and Chemical Engineers, approved by the Supervisory Committee on Standard Methods of Analysis of the American Chemical Soc., Journ. Ind. and Eng. Chem., Sept. 1911, 684.

pared silver carbonate; for each test, precipitate 140 mls of 0.5 percent silver nitrate solution with 4.9 mls of normal sodium carbonate solution; allow the precipitate to settle, decant and wash once with 150 mls of distilled water, by decantation; (5) solution of lead subacetate; boil a ten per cent solution of lead acetate with excess of litharge (2 parts to 3 of lead acetate) one hour, keeping the volume constant by addition of distilled water and filter hot (keep in a bottle from which  $\text{CO}_2$  is excluded); (6) a freshly prepared solution 1:1000 of potassium ferricyanide.

**158. Assay process.** Weigh accurately but rapidly about 20 gm. of the glycerin, dilute to 250 mls and take for the assay 25 mls of the dilution. Add the silver carbonate, shake occasionally during 10 minutes, then add 5 mls of the solution of lead subacetate. After 5 minutes dilute with distilled water to 100 mls, add further 0.15 ml of water to make up for the volume of the precipitate, mix thoroughly, filter through a dry filter into a narrow necked flask, rejecting the first 10 mls of filtrate (returning the filtrate if necessary until perfectly clear). (Test a portion of the filtrate with a few drops of the lead subacetate; should a precipitate appear, take another aliquot of 25 mls of the diluted glycerin, and proceed as before, using 6 mls instead of 5 of the lead solution). Into a flask which has been previously cleaned with potassium dichromate and sulphuric acid and drained, measure 25 mls of the filtrate, add 12 drops of diluted sulphuric acid (1:4) to precipitate any excess of lead present, then add 3.7282 gm. of the powdered pure potassium dichromate. Rinse down with 25 mls of water, and let stand with occasional shaking until the whole of the dichromate is dissolved. Add 50 mls of 50 percent sulphuric acid (by volume) and immerse the flask in a boiling water bath two hours, excluding access of dust or organic vapors such as that of alcohol. Add from a weighing bottle a slight excess of ferrous ammonium sulphate, making spot tests on porcelain

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\*Note that the 3.7282 gm. of potassium dichromate used corresponds with 0.500 gm. of glycerol, and that each ml of the standard dichromate solution corresponds with 0.01 gm. of glycerol.

with the potassium ferricyanide solution. Titrate back with the standard potassium dichromate solution and from the amount of dichromate reduced, calculate the percentage of glycerin, each gramme of the dichromate corresponding with 0.13411 gm. glycerol. (In applying this method to glycerin practically free from chlorides, reduce the silver carbonate to one-fifth the quantity prescribed above, and the solution lead subacetate to 0.5 mil. To secure a clear filtrate, it is sometimes necessary to add a little potassium sulphate.)

**159. Acetin method of Benedikt and Cantor,\*** modified.† This is based on the fact that on heating glycerol with acetic anhydride, acetin (tritenyl acetate) is formed. The acetin is treated with volumetric sodium hydroxide solution which combines with the potential acetic acid, the excess of alkali being determined by titration with volumetric acid. The method finds general favor on account of its comparative freedom from error due to presence of impurities, but when applied to a high grade glycerin, it has little if any advantage in exactness over the dichromate method, which is rapid and requires no special apparatus, and which consequently has been more commonly employed. The acetin method is likely to be accepted as the standard method. Heretofore it has been advised that both these methods be used, the mean of the results being adopted. It has been observed, however, that when there is any considerable discrepancy in the results of the two methods, that reached by oxidation with potassium dichromate is as a rule the higher, but also that the acetin result is high. If the difference is very great the acetin determination is to be accepted as approximately correct. When the difference is small, we may deduct from the acetin result one half the difference between the two results as the most probable figure.

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\*Journ. Soc. Chem. Ind., 1888, 696.

†Report of sub-committee of American Institute of Chemical Engineers, approved by a committee of the American Chemical Society, Journ. Ind. & Eng. Chem., Sept. 1911, 683.

**160. Assay Process.** Introduce into a tared narrow mouthed flask, capacity about 120 mls, about 1 ml of the glycerin, (which should contain not to exceed 60% of water), and weigh accurately but rapidly. Add about 3 gm. of pure recently fused anhydrous sodium acetate, then 7.5 mls of pure acetic anhydride, connect the flask with an upright reflux condenser, preferably by a ground glass joint; if a rubber stopper is used, it must be specially prepared by exposure to vapor of boiling acetic anhydride. Maintain at a boiling temperature one hour, being careful to prevent the salts from drying on the sides of the flask. Allow the flask to cool somewhat, then add through the condenser tube 50 mls of distilled water, free from  $\text{CO}_2$ , previously heated to  $80^\circ \text{C}$ . Maintain this temperature, without exceeding it, until solution of the salt is complete.

**161.** Without disconnecting the flask, allow it to become quite cold, then wash down the tube of the condenser with water, detach the flask, rinse off stopper or ground glass joint into the flask and filter the solution into a Jena glass flask of about 1 liter capacity. Wash the filter well with cold distilled water free from  $\text{CO}_2$ , add 2 mls of a neutral solution (1:200) of phenolphthalein in alcohol, and neutralize cautiously with normal sodium hydroxide free from carbonate, taking care to shake constantly while adding the reagent until the color just changes to faint pinkish yellow. By no means allow the color to become a full pink, otherwise acetin will be prematurely hydrolyzed and results of the assay will be low. Now add exactly 50 mls of normal sodium hydroxide (free from  $\text{CO}_2$ ) and boil the solution gently 15 minutes, the flask being fitted with a glass tube to act as a partial condenser. Cool as rapidly as possible and titrate excess of sodium hydroxide with normal acid to production of the same pinkish yellow tint as before. Subtract the volume in mls of normal acid used from 50 and multiply the remainder by 0.03069 to find the quantity of glycerol in the sample taken for assay.

162. **A blank test** should be run to ascertain whether there is present in the reagents used any impurity which would seem to indicate presence of glycerol, and a corresponding correction must be made if necessary. If the glycerin leaves a residue when evaporated at  $160^{\circ}\text{C.}$ , this must be dissolved in a little water, transferred to the acetylation flask, evaporated to dryness, acetic anhydride and sodium acetate added, and the acetin value of the residue in terms of glycerol determined exactly as above (applying to the result the same correction as before for the blank). This finally is to be deducted from the result of the first determination for actual glycerol in the sample.

163. **Example.** Weight of the glycerin taken for the assay (1 mil), 1.2435; result of first assay, glycerol 1.1953; result of blank 0.0087, result of assay of residue not volatile at  $160^{\circ}\text{C.}$ , 0.0912.

Total apparent glycerol,  $1.1953 - 0.0087 = 1.1866$

Apparent glycerol in residue,  $0.0912 - 0.0087 = 0.0825$

Corrected result  $1.1866 - 0.0825 = 1.1041\text{ gm.}$

Calculated to percent = 88.79

164. **Permanganate oxidation process** (suggested by Wanklyn, improved by Fox, Benedikt, Zsigmondy, Allen and others)\*. To about 0.25 gm. (accurately weighed) of glycerin, which must be free from ethyl alcohol, add 250 mls of a 4 percent solution of potassium hydroxide and, under continuous shaking, 6 mls of a 5 percent solution of potassium permanganate. After half an hour, add solution hydrogen dioxide sufficient to completely decolorize the liquid. Make up to 1 liter, shake well and filter through a dry filter. Heat 500 mls of the filtrate for half an hour to destroy excess of hydrogen dioxide, cool to about  $60^{\circ}\text{C.}$  and titrate the oxalic acid present with tenth-normal potassium permanganate. Each mil of this solution corresponds with 0.004502 gm. of anhydrous oxalic acid, or 0.004603 gm. of glycerol.

\*Mangold in Journ. Soc. Chem. Ind., 1891, 803.

**165. Hydriodic Acid process.\*** The sample is distilled in a special apparatus with a solution of hydriodic acid (57 to 63 percent) in a current of carbon dioxide. The vapors, cooled to 60°, are passed through a small wash bottle containing amorphous phosphorus, suspended in water at 60° to free them from iodine or hydrogen iodide (Stritar substitutes for the red phosphorus, sodium and antimonyl tartrate). The isopropyl iodide formed is passed through a 4 percent alcoholic solution of silver nitrate and the silver iodide produced is collected and weighed. Its weight multiplied by 0.3922 gives the amount of glycerol in the sample.†

**166. Method by distillation.** L. C. Janssens‡ proceeds as follows: Fill one side (A) of a U-tube with copper turnings and pack the other side (B) loosely with asbestos, which is made to absorb a weighed portion of the slightly alkaline glycerin. Cover this with a small plug of asbestos, place the U-tube in a paraffin bath heated to 200° C., connecting B with a condenser and receiver. Pass a current of steam into A and distil until from 10 to 20 times the volume of the crude glycerin has been collected. Determine the glycerol in the distillate by specific gravity, by use of the refractometer or by any of the approved methods of assay.

**167. Vacuum distillation with Oil Sandalwood,** applicable especially to estimation of glycerin in complex mixtures such as tooth-pastes, face creams, etc.\*\* Results of a great degree of exactness are not to be expected by this method. A quantity of the material to be assayed which will yield approximately

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\*Zeisel and Fanto, Zeit. landw. Versuchs-Wes. Oester., 4,977 and 5,729.

†For simplified form of apparatus for this assay see Trans. Chem. Soc., p. 318. Fr. Schultze (Chem. Ztg., 1905, 29, 976-980) after a critical study of the various methods of determining glycerol, concludes that that of Zeisel and Fanto is the only one which is trustworthy.

‡Chem. Centralbl., 1906 II, 273, from Seifensiederzeit., 33, 286.

\*\*C. H. Briggs in Journ. Am. Pharm. Assoc., Jan. 1915, 75.



2 gm. glycerol is placed in a 500 mil side-neck distillation flask with 0.5 gm. of calcined magnesia and the mixture heated 5 minutes on a steam bath; 75 mls of sandalwood oil is added and the apparatus arranged for vacuum distillation. Distil until about 50 mls of the oil has passed over, disconnect the receiver, rinse the condenser with 100 mls of purified petroleum benzin and then with 5 mls of distilled water, adding these to the distillate. Shake, separate and draw off the aqueous layer into a second separator. Shake out the benzin solution with three additional portions of water (5 mls) then shake the combined aqueous solutions with 30 mls of petroleum benzin. After half an hour, draw off the water into a 10 cm. petrie dish, shake the benzin with 4 mls of water, which after separation is to be added to the solution in the dish. Evaporate this solution at a temperature below 50° C. and finally dehydrate in vacuo over sulphuric acid and weigh as anhydrous glycerin.

**168. Method by direct extraction with acetone\*.** Take a quantity of the solution containing approximately 1 gm. of glycerol, concentrate by evaporation at a temperature below 50° C. to a syrupy consistence and mix with 20 gm. of powdered anhydrous sodium sulphate. Transfer to a soxhlet apparatus and extract 10 hours with redistilled anhydrous acetone. After evaporating the solvent, dry the residue of glycerol to constant weight with the usual precautions.

**169. F. C. Cook† advises** to dissolve the crude glycerin in a little water, add "a little" silver nitrate, make up to 100 mls, let stand over night, filter and determine glycerol in an aliquot part of the solution by the dichromate method (See 158). The crude glycerin may perhaps be submitted with advantage to examination with the refractometer.

**170. Glycerin may be determined in supposi-**

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\*A. H. Shukoff and P. J. Schestakoff. Zeit. angew. Chem. 1905, 18, 294-5.

†Journ. A. O. A. C., Aug. 15, 1915, p. 379-81.

**tories**, according to C. G. Vanderkleed and F. Heidelberg\* by dissolving about 2 gm. of the sample in a separator with hot water acidified with sulphuric acid, cooling and shaking out with ether to remove stearic acid. The aqueous solution is concentrated by cautious evaporation, 10 mls of water are added and the evaporation is repeated to drive off traces of ether, and glycerol is determined by oxidation with potassium dichromate and sulphuric acid (155).

## METHYL ALCOHOL

**171. Because of the poisonous nature** of methyl alcohol, and the fact that its use in medicinal preparations is illegal, tests for the detection and estimation of this compound in mixtures are of especial importance. Many methods have been suggested for the detection of methyl alcohol, particularly in mixtures containing also ethyl alcohol. The first step is the separation by distillation of the alcohols from non-volatile matter. If free iodine is present it must be first removed by sodium thiosulphate. Volatile oils must be removed from the distillate by salting out and shaking with petroleum benzin, afterwards redistilling as is done in determinations of ethyl alcohol. Oxidation (by dichromate, permanganate, persulphate and similar agents) produces in case of ethyl alcohol (ultimately) acetic acid, in the case of methyl alcohol carbon dioxide, or in some cases formic acid. Either of these may be made a basis for a determination of the methyl alcohol. Incomplete oxidation yields aldehydes, that from methyl alcohol, formaldehyde, being easily recognized by familiar tests, or even by its odor.

**172. Quantitative determination** of either ethyl or methyl alcohol, in absence of other oxidizable substances, can be made by oxidation with potassium dichromate and sulphuric acid, the excess of potential oxygen being determined by adding potassium iodide

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\*Proc. Penn. Pharm. Assoc., 1912, 307-8.

and titrating the iodine set free. Inasmuch as the two alcohols differ greatly in the amount of oxygen required,\* it is possible, in a mixture of the two alcohols with water, first by the specific gravity of the mixture to determine approximately the proportion of the alcohols and second by the amount of oxygen consumed to determine the percentage of each of the alcohols.

173. Detection of methyl alcohol in distillates containing also ethyl alcohol. Perhaps the most satisfactory test is that of L. E. Hinkel.† Add to 1 mil of the mixture (distillate) 0.8 gm. ammonium persulphate and 5 mils of diluted sulphuric acid,‡ make up to 20 mils with water, distil 4 mils and reject as containing acetaldehyde, then collect 3 portions of 2 mils, add to each a few drops of a 0.5 percent solution of morphine hydrochloride and underlay the fluid with concentrated sulphuric acid. If methyl alcohol is present, a violet zone will appear at the plane of contact of the two fluids.

174. Test of Deniges,§ modified by E. Elvove\*\* (colorimetric). Use for the test 5 mils of a solution containing not more than 1 mg. in a mil of methyl alcohol. If this contains in each mil as much as 5 mg. of ethyl alcohol, no more of this need be added, otherwise add up to 1 mil of a 2.5 percent solution of ethyl alcohol. Add 2.5 mils of a 2 percent solution of potassium permanganate and 0.2 mil of concentrated sulphuric acid, and allow the mixture to stand 3 minutes. Then add 1 mil of a 6.7 percent solution of oxalic acid followed by 1 mil of concentrated sulphuric acid, cool to room temperature and mix with 5 mils of Schiff's reagent (fuchsin sulphurous

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\*One gm. of methyl alcohol requires 1.5 gm. of oxygen, while 1 gm. of ethyl alcohol requires only 0.695 gm. of oxygen.

†Analyst, 1908, 417-9.

‡In place of the ammonium persulphate and dilute sulphuric acid, potassium dichromate 1.5 gm. and strong sulphuric acid 1.5 gm. may be used.

§Compt. Rend., 1910, 832.

\*\*Journ. Ind. & Eng. Chem., 1917, 295-7;

acid test solution, U. S. P.). Let stand 40 minutes, then transfer to a 50 mil Nessler tube (narrow form) and compare with a solution prepared in the same manner but containing a known quantity (4 mg.) of methyl alcohol. (Standards should be made containing resp. 1, 2, 3, 4, and 5 mg. of methyl alcohol.) If the test is only qualitative for the detection of very small quantities of methyl alcohol, the time given for full development of the color must be increased to one or even two hours. To prepare the reagent, Elvove dissolves 0.2 gm. of finely powered fuchsin in 120 mls of hot water, cools to room temperature, adds a solution of 0.2 gm. of anhydrous sodium sulphite in 20 mls of water, then 2 mls of hydrochloric acid (sp. gr. 1.19) and water to make 200 mls; let stand an hour before using. Prepared in this way, the reagent will remain serviceable several weeks if kept in a well closed bottle.

175. When tinctures and similar preparations are tested merely qualitatively for traces of methyl alcohol, only the first portion of the distillate should be examined, since the boiling point of this compound is lower than that of ethyl alcohol.

176. **Application of Hehner's test.** Franz Lörinsch directs to add to 1 mil of the distillate from a tincture or fluid extract (171) 1 mil of 25 percent sulphuric acid and 8 mls of tenth-normal potassium permanganate solution. After 10 minutes filter and mix with 1 mil of a mixture of equal parts of milk and water, to which one drop of a solution of ferric chloride (4%) has been added, and underlay the mixture with strong sulphuric acid. If methyl alcohol is present a violet colored zone is produced at the plane of contact of the fluids. Another method of procedure is to add to 2 mls of the solution which has been treated with permanganate a drop of a ferric chloride solution (0.4%) and 40 mg. of peptone (or of powdered milk) then underlay the mixture with pure sulphuric acid. The test in this form will detect an admixture with a hydro-alcoholic fluid of 0.015% of methyl alcohol, or

of a much smaller proportion if the solution is first concentrated by fractional distillation.

**177. Application of Rimini's test for formaldehyde.** To 5 mls of a solution prepared as in the foregoing test, add 1 mil of a freshly prepared and filtered 2 percent solution of phenylhydrazine hydrochloride, after 2 minutes add 1 mil of a freshly prepared 4 percent solution of potassium ferricyanide and immediately 1 mil of hydrochloric acid. A cherry red color is produced which changes gradually (within 20 minutes) to a raspberry red. The color is strong when the solution tested represents 20 mg. of methyl alcohol, and if the volume of fluid tested is only 1 mil, 1 mg. of the alcohol gives a good test, but the depth of the color varies in an apparently capricious manner. There is room for a study of the conditions under which the color is most fully developed.

**178. Quantitative determination by oxidation to formic acid.** R. Schmiedel\* adds to the solution at a temperature of 5° C. a dilute solution of hydrogen peroxide, little by little. Methyl alcohol is oxidized to formic acid, ethyl alcohol to acetic acid. The acids are distilled off in a current of steam, neutralized with calcium carbonate, and the formic acid determined by its reducing action on corrosive sublimate. Each gm. of mercurous chloride formed corresponds with 0.0678 gm. of methyl alcohol. (Query: Is the methyl alcohol oxidized quantitatively to formic acid under the conditions prescribed?)

**179. Determination by complete oxidation.†** Fit a liter flask with a reflux condenser and also with a soda-lime absorption tube. Put into the flask some pumice which has been boiled with potassium permanganate and sulphuric acid and ignited. Connect the condenser with an absorption system consisting of U-tubes containing sulphuric acid, calcium chloride and soda lime, the last tared. One to 3 gm. of the alcohol to be tested is placed in the flask, followed by a mixture of potassium dichromate 30 gm., water 500

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\*Pharm. Zentr.-h., 1913, 709-16.

†W Koenig in Chem. Zeit., 1912, 1025-7.

mils, and strong sulphuric acid 50 mils, which has been boiled and cooled to 5° C. Let the mixture stand 4 hours at ordinary room temperature, then apply heat gradually until the solution begins to boil. Then draw air through the apparatus to sweep out every trace of carbon dioxide. The gain in weight of the tared U-tube is the weight of the carbon dioxide formed, each gm. of which corresponds with 0.728 gm. of methyl alcohol.

**180. Determination by the refractometer.** The specific gravities of mixtures of methyl alcohol and water are very nearly the same as those of mixtures of ethyl alcohol and water having the same percentage strength. The indices of refraction, however, of dilutions of the two alcohols differ widely. A. E. Leach and H. C. Lythgoe\* base on these facts a practical method of determining approximately the proportions of these two alcohols when present in aqueous dilutions, in absence of other substances. Although such a method can have only a limited application in practice, it merits notice here. The mixture is to be distilled and brought to its original volume at the temperature at which it was originally measured. From its specific gravity, the percentage by weight of alcohol (assumed to be ethyl alcohol) is found by alcoholimetric tables. The reading of a Zeiss immersion refractometer is taken at 20° C. From the appended table find what should be the reading if the mixture consisted simply of ethyl alcohol and water. Subtract from this the actual reading. Find in the third column of the table the percentage of methyl alcohol corresponding with an integral unit of this difference. Multiply this by the difference to find the actual percentage of methyl alcohol. Subtract this from the percent of ethyl alcohol shown by the alcoholimetric table to find the actual percent of ethyl alcohol.

**181. Table for determination of Methyl Alcohol in admixture with Ethyl Alcohol and water, by Zeiss Immersion Refractometer.**

<i>Percent Alcohol by Specific Gravity</i>	<i>Scale Reading Ethyl Alcohol</i>	<i>Percent Methyl Alcohol = Difference 1° Scale Reading</i>	<i>Percent Alcohol by Specific Gravity</i>	<i>Scale Reading Ethyl Alcohol</i>	<i>Percent Methyl Alcohol = Difference 1° Scale Reading</i>	<i>Percent Alcohol by Specific Gravity</i>	<i>Scale Reading Ethyl Alcohol</i>	<i>Percent Methyl Alcohol = Difference 1° Scale Reading</i>
1	16.0	0.833	36	76.9	0.886	71	100.2	1.046
2	17.6	0.908	37	78.0	0.896	72	100.4	1.047
3	19.1	0.958	38	79.1	0.906	73	100.6	1.048
4	20.7	0.976	39	80.2	0.916	74	100.8	1.050
5	22.3	0.981	40	81.3	0.926	75	101.0	1.052
6	24.1	0.960	41	82.3	0.934	76	101.0	1.056
7	25.9	0.940	42	83.3	0.941	77	100.9	1.060
8	27.8	0.919	43	84.2	0.948	78	100.9	1.064
9	29.6	0.900	44	85.2	0.955	79	100.8	1.068
10	31.4	0.893	45	86.2	0.962	80	100.7	1.072
11	33.2	0.887	46	87.0	0.968	81	100.6	1.075
12	35.0	0.882	47	87.8	0.974	82	100.5	1.078
13	36.9	0.877	48	88.7	0.979	83	100.4	1.081
14	38.7	0.872	49	89.5	0.985	84	100.3	1.084
15	40.5	0.867	50	90.3	0.990	85	100.1	1.085
16	42.5	0.861	51	91.1	0.995	86	99.8	1.086
17	44.5	0.853	52	91.8	1.000	87	99.5	1.088
18	46.5	0.847	53	92.4	1.005	88	99.2	1.089
19	48.5	0.840	54	93.0	1.010	89	98.9	1.090
20	50.5	0.833	55	93.6	1.015	90	98.6	1.091
21	52.4	0.830	56	94.1	1.019	91	98.3	1.093
22	54.3	0.827	57	94.7	1.022	92	97.8	1.095
23	56.3	0.824	58	95.2	1.025	93	97.2	1.097
24	58.2	0.823	59	95.7	1.027	94	96.4	1.099
25	60.1	0.823	60	96.2	1.029	95	95.7	1.103
26	61.9	0.824	61	96.7	1.031	96	94.9	1.107
27	63.7	0.825	62	97.1	1.032	97	94.0	1.111
28	65.5	0.827	63	97.5	1.033	98	93.0	1.115
29	67.2	0.829	64	98.0	1.034	99	92.0	1.120
30	69.0	0.832	65	98.3	1.035	100	91.0	1.124
31	70.4	0.840	66	98.7	1.036			
32	71.7	0.848	67	99.1	1.039			
33	73.1	0.857	68	99.4	1.041			
34	74.4	0.866	69	99.7	1.043			
35	75.8	0.875	70	100.0	1.045			

**182. Example.** The mixture has a specific gravity which according to an alcoholimetric table (e. g. that of U. S. P. IX, pp. 633-637) indicates 47.6 percent of (ethyl) alcohol. This according to column II of accompanying table should show a refractometer reading of 88.34 (by interpolation). In fact the reading is 65.5, a difference of 22.84. Column III gives (by interpolation) the factor 0.977, which multiplied by 22.84 (the difference above) gives as the percentage of methyl alcohol 22.31. This subtracted from total alcohol (47.6) gives as the percentage of ethyl alcohol 25.29. These figures of course are only approximate, since the specific gravity of dilutions of the two alcohols do not accurately coincide. Results should be checked by other tests, such as those of (176) or (178) applied to quantities of the sample which should show a minimum distinct reaction.



## Chapter II

### Alkaloidal Drugs

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#### ACONITE

183. No remedy of the *materia medica* stands more in need of a reliable basis of standardization than aconite. The alkaloid on which it depends for its therapeutic action is one of extraordinary potency, but it is not the only alkaloid present in the official drug, and it is replaced in other species of aconite, which have, notwithstanding, analogous medicinal properties, by alkaloids which have similar, yet not identical constitution. Leaving out of consideration unofficial species, such as the Japanese aconite, we still are confronted with the problem of separating aconitine from other alkaloids that may accompany it in the fresh drug, and the equally perplexing problem arising from the spontaneous splitting of aconitine into benzoic acid and the inert alkaloid aconine. The assay processes hitherto in use for this drug are as a rule content with determining merely the total content of alkaloid in the sample, this being assumed to consist essentially of aconitine. The molecular weight of this alkaloid being extraordinarily high, results obtained by alkalimetric titration are liable to be above the truth, and not infrequently greater than the actual weight of the crude alkaloid. That the assay processes of the several pharmacopœias lack scientific precision to a lamentable degree must be confessed. That they are wholly valueless, on the other hand, is an unwarranted conclusion.

184. Extraction of total alkaloids from the drug is a simple matter. Type process I (102) is commonly followed, using ether alone as the immiscible solvent. The molecular weight of the alkaloid is so high that

if it is both weighed and titrated, it is easy to see whether or not it consists mainly of aconitine. In no case should titration show a figure higher than the actual weight of the alkaloid, since this is proof that a part at least of the aconitine has suffered disintegration or at least that alkaloids other than aconitine are present. It is the foregoing method which is official in the U. S. P. IX. The drug is required to show by this assay a minimum of 0.5 percent ether-soluble alkaloid, each mil of tenth-normal acid corresponding with 0.06454 gm. of aconitine. The British Pharmacopœia, using an assay method resembling that of (105), requires a minimum of only 0.4 percent.

**185. Assay process of E. H. Farr and R. Wright.\*** Exhaust 20 gm. of aconite root in coarse powder by percolation with 70 percent (vol.) alcohol. Evaporate this tincture on the water bath to a small bulk, add 15 mls of tenth-normal sulphuric acid, and water to make 40 mls, filter, and wash residue with acidulated water. Treat the united filtrate and washings with chloroform (15 and 15 mls) to remove coloring matter. Wash the chloroform with 10 mls of acidulated water to recover traces of alkaloid. Treat the mixed acid solutions with potassium carbonate in slight excess and shake out the alkaloids with chloroform (35 and 35 mls). Wash the chloroformic solution with a little water. Evaporate and dry to constant weight at 100° C.

**186.** Aconitine yields by saponification a definite quantity of benzoic acid, and this has been made by A. H. Allen† the basis of assay processes for aconite and its preparations. The alkaloid extracted by (185) or other method (not more than 0.08 gm.) is treated with 20 mls of 70 percent alcohol and 3 mls of a 50 percent solution of sodium hydroxide. The mixture is boiled one hour under a reflux condenser, the alcohol is then evaporated off and the solution acidulated with hydrochloric acid. The benzoic acid pro-

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\*Pharm. Journ., [3] XXI, 1037.

†Commercial Organic Analysis.

duced by the saponification is extracted by shaking out with ether (15 and 10 mls), the ether is washed with water until the washings no longer redden litmus paper, the ether is titrated with fiftieth-normal barium hydroxide (phenolphthalein indicator) to production of a permanent pink color. Each mil of fiftieth-normal barium hydroxide corresponds with 12.94 mg. of aconitine (resp. with 12.44 mg. of japaconitine, or with 14.14 mg. of pseudaconitine).

**187. The Silico-tungstic acid method\*** of H. Ecalle.† Put into a 250 mil stoppered bottle or flask fifteen gm. of aconite in No. 40 powder, and 150 mls of ether. Stopper the bottle, shake well, let stand ten minutes, then add 5 mls of ammonia water, stopper the bottle and shake vigorously at frequent intervals during two hours, or preferably continuously with a mechanical shaker. Add 15 mls of water (or a sufficient quantity), shake well and set by for 15 minutes, then decant rapidly through absorbent cotton 100 mls of the ether solution, transfer this to a separator, and follow thereafter exactly the instructions given in the French Codex (1908) as follows: Add 10 mls of diluted nitric acid (10 percent) and 10 mls of distilled water, shake well and let separate. Draw off the acid solution into a 250 mil beaker, shake out the ether with four successive portions (20 mls) of distilled water, which is to be added to the acid solution in the beaker. Heat on a water bath to expel traces of ether; after cooling add 15 mls of a five percent solution of silicotungstic acid followed by 10 mls of diluted nitric acid. Heat the mixture to boiling, then set it aside for twenty-four hours. Collect the precipitate on a plain filter and wash it with distilled water (cold) until the washings no longer redden litmus paper. Dry the precipitate, ignite and weigh. Multiply the weight by 0.793 to find the weight of the aconitine contained in 10 gm. of the aconite.

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\*Substantially the method official in the French Codex.

†H. Ribaut (1911) reports that the method carried out thus gives trustworthy results when applied to solutions of pure aconitine. *Journ. Pharm Chim.* (6) 14,97.

188. **For the fluidextract** and the tincture of aconite, the U. S. P. IX employs the sawdust expedient (112), although there seems to be really no necessity for this. Cheese cloth may be substituted for the sawdust (113). The standards fixed for these preparations correspond with those for the crude drug, and the same is true of the B. P. tincture (1914), the strength of which now approximates that of the international standard (0.05 percent).

189. Method (for tincture of aconite) published in Caesar & Loretz' *Geschäfts-Bericht*, 1903. Evaporate in a beaker with frequent stirring 100 mls tincture of aconite to about 15 mls. Transfer to a separator. Rinse the beaker after adding 1 drop of ammonia water with 25 mls of ether, and add this to the separator, avoiding evaporation as far as possible. Add further 76 mls of ether and 1 ml of ammonia water, stopper and shake frequently during 15 minutes, let stand 15 minutes, then decant rapidly through absorbent cotton 80 mls of the ether. Evaporate (or distil) to about 10 mls, add 5 mls dehydrated alcohol, 30 mls ether and 3 drops of haematoxylin indicator, then titrate with tenth-normal hydrochloric acid until the watery layer is reddish brown, add 30 mls of water and continue titration until the color changes to lemon yellow. Each mil of tenth-normal acid corresponds to 0.06454 gm. aconitine, the quantity thus found being that contained in 80 mls of the tincture.

190. The chemical assay of aconite by methods commonly practised being confessedly of questionable value, we are left the alternative of a **biological assay**. Dr. E. R. Squibb many years ago\* worked out an empirical biological method which has been rather widely practised. The test depends on the characteristic tingling produced on lips and tongue by a highly diluted solution of aconitine. Prepare an aqueous solution 250 mls of which represent 1 gm. of aconite root. Dilute a portion of this with twice its volume of water. After rinsing the mouth well with water,

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\**Ephemeris*, p. 125 et seq.

take into the anterior part of the buccal cavity 4 mls of the diluted solution and hold it there exactly one minute, then reject it and rinse the mouth once more with water. If the aconite is of normal strength, the characteristic sensation of numbness and prickling will be produced, beginning within 15 minutes and lasting as much as 1.5 hours. If the sensation is not experienced, or only faintly, make a second experiment diluting the original strong solution 1:1.5 or 1:1. At least 2 hours [preferably not less than 12 (Ed.)] should intervene after the sensation has passed away before the second test is made. If the sensation produced in the first trial is very pronounced or lasts longer than 1.5 hours, use for the second trial a dilution of 1:2.5 or 1:3. Continue the tests until a dilution is found which produces the effects above described as normal. State the conclusion in terms of degree of dilution of the solution which produces a normal reaction, e. g. 1:800 if the solution represents in 800 volume parts, 1 part by weight of aconite. This strength, as a matter of fact, is about what may be accepted as standard.

190½. Obviously a test like this can serve the purpose only of comparing one sample of drug with another, since individuals vary greatly in susceptibility to the aconite impression. At best, the test is only very roughly quantitative. Unless two samples are directly compared under conditions as nearly as possible identical, variations as high as twenty percent may be expected. At the same time, a preparation of aconite should certainly produce on at least 2 out of 3 subjects, taken at random the effect above described when tested in dilutions for the drug or fluidextract of 1:750; for the tincture of 1:75; for the extract of 1:3000.

191. **A recent study of the Squibb test** by the author in collaboration with Wilbur L. Scoville and some of his associates has led to some rather interesting conclusions: (1) In place of water, a physiological-salt solution is advantageously used in making the dilutions. (2) Not more than one test should be

made in 24 hours, to avoid cumulative effects. (3) The solution to be tested should not be held in the mouth more than 15 seconds; perhaps 10 seconds is to be preferred. The reason is that the effect produced is not in proportion to the duration of the experiment. Dilution of the solution by the saliva partly accounts for this. (4) It is better to take 8 or 10 than 4 mils of the dilution for a test, since the effect of the added salivary secretion is not so great. (5) In case of highly dilute solutions, e. g. 1:3000, a distinct tingling sensation is perceived within a minute or two, lasting 10 to 15 minutes. If the dilution is 1:2000, the tingling sensation is not so certainly produced, and does not come on so promptly. A stronger solution produces still less of the characteristic tingling, the effect seeming to be primarily to paralyze the nerves. The sensation finally produced is one rather of numbness than of tingling, and it is likely to last an hour or more. (6) No method of making the test gives anything approximating quantitative results, but something is to be hoped from a further careful study of the effects produced by dilutions of 1:3500 and upwards. It may turn out that the vanishing point for the reaction under just the right conditions may be somewhat sharply defined.

#### 192. Official biological test of U. S. P. IX.

The test consists in determination of the minimum lethal dose of the drug for a guinea pig. The drug in solution is injected subcutaneously into healthy animals weighing 250 to 300 gm., the exact weight being noted in each case. The minimum quantity which causes death within twelve hours is ascertained in a series of experiments. The standards of the U. S. P. for minimum lethal dose per gramme body weight are, for fluidextract aconite root, 0.00004 mil; for tincture, 0.0004 mil; for extract, 0.00001 mil.

193. It is maintained by some that the susceptibility of guinea pigs varies under different conditions so that results of the U. S. P. assay cannot be trusted. Cats have been substituted, and where only the male animal is used, it is stated that results are more self-consistent. The gold-fish method may prove to be

preferable to either of the foregoing. It must be confessed that as yet a reasonably exact standard for preparations of aconite remains a desideratum. The criticism to be made of all of these methods of assay is that they give no information with regard to the action of the drug in medicinal doses, which may have no quantitative correspondence with the overwhelming power of a lethal dose. Obviously scientific standardization of a drug by the biological method must be based on knowledge of the symptoms attending the therapeutic action of the drug, such symptoms being capable of reduction to quantitative expression, e. g. increase or diminution of blood pressure.

## MYDRIATIC DRUGS

**194. Belladonna root and leaf, Stramonium leaf and seed, and Hyoscyamus** may be considered under one head, since they contain identical or closely related alkaloids (atropine, hyoscyamine). Scopola may be added to the list, although this drug contains in larger proportion than the others, the alkaloid scopolamine (hyoscine). The same assay processes are applicable to all. The alkaloids are easily determined with precision by titration with volumetric acid, but we have to bear in mind that they are rather easily hydrolysed, and hence in the choice of assay methods we have to avoid the prolonged application of heat, or the use, except with caution, of such agents as the caustic alkalies. Hyoscyamus contains so little alkaloid that it is necessary to use a large quantity of drug and a correspondingly liberal amount of volatile solvent. See (329)—(333).

**195. Results of the assay** are reported in terms of atropine for all the drugs of this group except scopola, for which results are given in terms of scopolamine. For the former the titration equivalent of 1 mil of tenth-normal acid is 0.028919, for the latter, 0.030318. Alkalimetric titration of these alkaloids is very satisfactory, their alkalinity being more pronounced than that of most alkaloids.

**196. Assay process for the mydriatic drugs** (type process I). Put into a 250 mil flask 15 gm. of the finely powdered drug (in the case of hyoscyamus 25 gm.) with 150 mls of a mixture, previously cooled to room temperature, of 1 volume of chloroform and 5 volumes of ether. After 10 minutes add 5 mls of water of ammonia, stopper the flask and shake vigorously every 10 minutes during 2 hours, then add, for belladonna or scopola root 15 mls, for belladonna, stramonium or scopola leaves 25 mls, for hyoscyamus 40 mls of water (enough in each case to cause agglutination of the drug powder), shake, add 1 gm. of powdered tragacanth, shake once more, then decant rapidly, avoiding evaporation, 100 mls of the ethereal solution, representing two thirds of the drug taken. Filter through absorbent cotton into a separator, wash the measuring flask and cotton with two portions (8 and 3 mls) of ether, adding this to the separator. Shake out with 4 portions (8 mls each) of dilute sulphuric acid (2 percent). Combine the acid solutions in a separator, wash with 10 mls of chloroform (to be rejected) add a bit of red litmus paper and water of ammonia sufficient to render the solution alkaline. Shake out the alkaloid with chloroform (25, 20, 20, 15 mls). Evaporate off the chloroform by a gentle heat, redissolve the alkaloid in a little alcohol and evaporate, repeating this treatment once more to remove all traces of chloroform. Finally dissolve the alkaloid in 3 mls of tenth-normal hydrochloric acid and titrate the excess of acid according to (70). Each mil of tenth-normal acid neutralized by the alkaloid corresponds with 0.02892 gm. of atropine or 0.03032 gm. of scopolamine.

**197. Assay process of German Pharmacopoeia.** The powdered drug is treated with ether and solution of sodium hydroxide, an aliquot of the ether is distilled to one third, the remainder is shaken out with weak hydrochloric acid. From the acid solution the alkaloid is extracted by making alkaline with sodium carbonate and shaking out with chloroform, the chloroform is treated with 20 mls of hundredth-normal hydrochloric acid and the excess of acid is



determined by titration with hundredth-normal potassium hydroxide. The foregoing is only an outline; the details of the assay are minutely circumstantial, yet the results are said to be inexact.

198. **The Swiss Pharmacopoeia** directs to macerate the drug with diluted alcohol one hour, with frequent shaking, to evaporate an aliquot of the filtered solution to a small volume, add some diluted hydrochloric acid, filter again, shake an aliquot of the filtrate (after addition of ammonia) with ether 15 minutes, evaporate an aliquot of the ether, treat residue with a little ether and again evaporate to dryness, finally titrate the alkaloidal residue with hundredth-normal hydrochloric acid. (The three successive aliquots, two of them of only approximate exactness, together with the very small quantity of drug represented in the final titration, make accuracy of results out of the question. It is fair to say that in the Swiss process for assay of *extract* of belladonna an aliquot is taken only once.)

199. **Determination of atropine by silicotungstic acid.\*** Dissolve the crude alkaloid (e. g. from a belladonna assay) in 500 times its weight of water containing 2 percent of hydrochloric acid. Add with constant stirring a slight excess of a solution of silicotungstic acid (10 percent). After 24 hours collect the precipitate on an ashless filter, wash it with 1 percent hydrochloric acid, dry, ignite and weigh. Multiply the weight by 0.4064 and add for each 100 mls of the mother liquor 0.0048 gm. to find the weight of atropine present. Results are said to be lower than those of alkalimetric titration. (The moist precipitate may be shaken out with ammonia and chloroform or ether, yielding the alkaloid in approximately pure condition.) See type process IV (105).

200. **The Potassium-Bismuth Iodide** method of Thoms† is carried out in the following manner, in case of extract of belladonna. Dissolve 2 gm. of the sample in 50 mls of water, and 10 mls of 10 per

\*Maurice Javilier, Bull. Sci. Pharmacol., 17, 629-34.

†Journ. de Pharm. et Chim., 1905, XXI, 605.

cent sulphuric acid, add 5 mls of the potassium-bismuth iodide reagent (see 96) with constant stirring, collect the precipitate and complete the assay as described in (96).

201. Determination of atropine by **Wagner's Reagent** (method of Gordin and Prescott). Follow details of (84).

**202. Assay process of Dunstan and Ransom.\***

(1) For Belladonna root. Exhaust 20 gm. of the dry and finely powdered drug by hot percolation (preferably by hot repercolation) with a mixture of chloroform and absolute alcohol, equal volumes. (For hot repercolation use 60 mls of the mixture.) Agitate the percolate with 2 successive portions (20 mls) of distilled water, which are separated in the usual way carrying with them the alkaloid. (Personally I should add 5 mls of 5 percent sulphuric acid before making the foregoing separation.) Shake out the aqueous solution with 15 mls of chloroform to remove impurities, then render it alkaline with ammonia and shake out the alkaloid with several successive portions of chloroform (25 mls), wash the separated chloroform with a little ammoniated water, transfer to a tared beaker and evaporate to dryness. Dry to constant weight and weigh the alkaloid.

203. (2) **For Belladonna leaves.** Exhaust 20 gm. of the dried and finely powdered drug by hot repercolation with absolute alcohol, of which about 100 mls will be required. Add to the percolate an equal volume of water with a little hydrochloric acid. From the slightly warmed liquid remove chlorophyll, fatty matter, etc. by repeated shaking out with chloroform, which may be continued as long as anything is removed by that solvent. Finally make the solution alkaline with ammonia and proceed as in the assay of belladonna root above.

204. **Assay method of A. F. Sievers.†** Used especially where only small quantities of the drug (belladonna) are at hand. Place in a Squibb's separa-

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\*Pharm. Journ. and Trans., 1884, 923, and 1885, 237.

†Journ. Am. Pharm. Assoc., 1912, 199.

tory funnel 2 to 5 gm. (accurately weighed) of the sample in fine powder, add 50 mls of a mixture of chloroform 1 volume, ether 4 volumes, shake, after 10 minutes add for each gm. of drug 1 mil of water of ammonia, shake at frequent intervals during 1 hour, draw off the ethereal solution through a pledget of absorbent cotton, then adjust the stopcock so as to deliver very slowly and percolate the mass with 60 mls of the same ether-chloroform mixture. Extract the alkaloid in the usual manner, by shaking out first with dilute acid, then with ammonia and chloroform.

**205. Assay of fluidextracts** of mydriatic drugs. Use type process A (110), extracting the alkaloid throughout with chloroform. In case of fluidextract of hyoscyamus, take for the assay 25 instead of 10 mls. Identical results may be reached by type process B\* (111). It is well to make one assay by each of the two type processes. It is also a good plan to purify the alkaloid obtained by type process B by dissolving it in tenth-normal sulphuric acid, washing the acid solution with several successive portions of chloroform (8 mls), then rendering alkaline and shaking out with chloroform. Weigh and titrate the alkaloid thus purified.

**206. Simplified assay for the pharmacist.**† Place in a separator 10 mls of the fluid extract, diluted sulphuric acid 10 mls, warm water 40 mls and chloroform 20 mls, shake, let separate and draw off the chloroform into a second separator; repeat the extraction with 10 mls of chloroform, wash the mixed chloroform solutions with 5 mls of water and 1 mil of diluted sulphuric acid. When separated, draw off and reject the chloroform solution, and transfer the aqueous solution to separator No. 1. Add to separator No. 1, water of ammonia in excess, and shake out the alkaloid with three successive portions (10 mls) of chloroform. The chloroform solution may be evaporated and the alkaloid determined by titration, or else the alkaloid may be extracted by shaking out with acid, then rendering alkaline and shaking out

\*A. B. Lyons in *Phar.n. Rev.*, Jan. 1903, 22-4.

†H. W. Gadd and S. C. Gadd, *Pharm. Jour.*, 1905: 438.

with chloroform, when it will be sufficiently pure to weigh.

**207. Assay of extracts of mydriatic drugs.** A pilular (so-called "solid") extract (2 to 4 gm.) is to be dissolved in the smallest practicable quantity of water by aid of 1 or 2 mls of water of ammonia, the solution to be assayed by type process A (110). Otherwise, bring the extract into aqueous or alcoholic solution as the case may be, as directed in (123) or (124). Perhaps the most satisfactory routine method is that in which lead subacetate is used, as in type process B for fluidextracts. Use 4 gm. of the extract, dissolved first in a little diluted alcohol and then made up with distilled water containing 5 to 10 mls of solution of lead subacetate, to 200 mls. Shake the mixture at intervals during 20 minutes, filter, to the filtrate add excess of sodium phosphate in powder (about 1.5 gm.). Filter once more and extract the alkaloids from 100 to 150 mls of the filtrate by shaking out with chloroform after addition of ammonia. The alkaloid will contain impurities, but can be determined quite accurately in the usual manner by residual titration.

**208. A powdered extract** is most conveniently assayed by placing in an Erlenmeyer flask 2 to 4 gm. of the powder, according to amount of alkaloid likely to be present, adding 100 mls of ether, shaking, adding 2 mls of water of ammonia, shaking the mixture at intervals during 20 minutes. Let stand until the ether solution is quite clear (if necessary add a little powdered tragacanth to clarify) then decant 80 mls of the ether solution, representing 80 percent of the quantity of powdered extract taken. Evaporate nearly to dryness, shake out with several successive portions (10, 5 and 5 mls) of 2 percent sulphuric acid wash the acid solution twice with chloroform (10 mls), then make alkaline with water of ammonia and shake out the alkaloid with chloroform in the usual manner. (In the U. S. P. assay, sand is mixed with the powdered extract, possibly with some advantage. The solvent employed there is a mixture of chloroform 1 volume and ether 2 volumes, having very nearly

the specific gravity of water. The proportions should be either 2:3 or 1:3, to make separations easy. Pure ether is to be preferred to either; pure chloroform may be employed advantageously if the sand is omitted.)

209. Method of H. Thoms\* by **Potassium-Bismuth Iodide** Dissolve 2 gm. of extract of belladonna in 50 mils of water and 10 mils of 10 percent sulphuric acid, add with constant stirring 5 mils of the reagent (96), collect the precipitate on a filter and wash twice with 10 percent sulphuric acid (5 mils); place the drained filter with the precipitate in a stoppered flask and treat with 0.3 gm. of sodium sulphite and 50 mils of solution of sodium hydroxide (15 percent). Shake well several minutes, add 5 gm. of coarsely powdered sodium carbonate (cryst.) and 100 mils of ether and shake well. After separation, pipette off 50 mils of the ether, representing 1 gm. of the extract, and titrate with hundredth-normal hydrochloric acid with iodeosin as indicator.

210. **Assay of Belladonna Plaster.** If spread on cloth, cut the spread plaster in strips and weigh it accurately. Dissolve it off the cloth in chloroform, using three successive portions. Wash the cloth with 80 mils of alcohol containing 1 mil of water of ammonia, pour the washings into the chloroform solution and stir the mixture until the rubber collects in a compact mass. (The cloth is to be dried and weighed and the weight deducted from that of the original spread plaster.) Transfer the chloroform-alcohol solution to a separator, rinse the container and rubber with 10 mils of alcohol, which is to be added to the separator. Follow this with 100 mils of water, rotate and let separate. Draw off the chloroform into a second separator and wash it with 50 mils of water, which is transferred to the first separator and washed there, along with the water first added, with fresh chloroform (10 and 5 mils) which is mixed with the first chloroform solution. Extract the alkaloid from the united chloroform solutions by shaking out repeatedly with weak sulphuric acid, and finally from

\*Journ. de Pharm. et Chim., 1905, XXI, 605.

the acid solution by shaking out with ammonia and chloroform. Details of the assay will be found in the original paper of A. H. Clark,\* also in U. S. P. IX.

**211. Assay method of U. S. P. VIII.** While the foregoing assay process seems to be an improvement on that official in U. S. P. VIII, preference for the latter is given by some of those who have had wide experience in this line. The method is as follows: Cut the spread plaster into strips, weigh it accurately, place it in a beaker with 50 mils of chloroform and 3 mils of water of ammonia. Stir until the plaster is wholly removed from the cloth. Decant the chloroform into another beaker, wash the cloth with 25 mils of chloroform and 1 mil of water of ammonia, and if necessary once more with 25 mils of chloroform. Unite the chloroform solutions (wash once with 20 mils of water to remove excess of ammonia), add alcohol 80 per cent of the total volume, stir and leave at rest until the rubber has separated into a compact mass. Transfer the solution to a separator and shake out with successive portions (20 then 10 mil) of half normal sulphuric acid until all alkaloid is extracted. Add to the united acid solutions an excess of ammonia and shake out the alkaloid with chloroform, finishing the assay in the usual manner. The cloth from which the plaster has been dissolved is dried and weighed and its weight deducted from the original weight of the sample.

**212. Per cent of alkaloid** contained in the several mydriatic drugs. Belladonna root as it has heretofore come into the American market, has contained from 0.3 to 0.8 percent of alkaloid, average about 0.5 percent. Belladonna leaves have contained 0.2 to 0.5, average 0.33 percent. The proportion of alkaloid has varied from season to season, so that it has been sometimes impossible to obtain drug of full official strength. The drug is coming to be cultivated in America to a limited extent, and there is reason to expect that American grown belladonna will under intelligent culture be uniformly of superior quality. By selection of seed from plants unusually rich in

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\*Proc. Am. Pharm. Assoc., 1910, 852-4.

alkaloid, it has been possible already to produce leaves yielding more than 1.2 percent of alkaloid. California grown belladonna is already coming into the market averaging more than 0.60 percent, even the stems sometimes containing 0.3 percent and over, and drug of equally good quality is reported from Michigan and other eastern states.

213. **Stramonium leaves** (commercial) yield 0.2 to 0.4 per cent alkaloid; average 0.25, the proportion varying with the season. Stramonium seed contains about the same proportion of alkaloid as the leaves.

214. **Hyoscyamus leaves** hardly average the official content of 0.065 of alkaloid. Often the proportion is less than half that. Probably under cultivation it could be greatly improved. An Egyptian grown henbane, not the product of *Hyoscyamus niger*, is very rich in alkaloid, yielding 1.25 to 1.50 percent. A large proportion of this is hyoscyamine.

## CACAO

215. **Determination of total xanthine bases** (caffeine and theobromine) in cacao or chocolate may be made according to Fromme,\* who has examined critically the methods of Mulder, Süss, Beckurts, Dekker and Wellmans, in the following manner: Boil 6 gm. of powdered cacao (or 12 gm. of powdered chocolate) in a tared liter flask under a reflux condenser with a mixture of 197 gm. of water and 3 gm. of diluted sulphuric acid. Add a mixture of 400 gm. of water with 8 gm. of calcined magnesia and continue the boiling one hour under the reflux condenser; when cool, make up any loss of water by evaporation, allow the mixture to settle and filter off 500 gm. (= 5 gm. cacao or 10 gm. chocolate) evaporate to the consistence of an extract, transfer by aid of 10 mls of water to a separator and shake out 8 times with hot chloroform, in 50-mil portions. Evaporate or distil off the chloroform and dry the residue (caffeine and theobromine) at a temperature not exceeding 80° C. to constant weight and weigh. Extraction of the alkaloids with

\*Apoth. Zeitung, Aug. 26, 1903, 593-6.

chloroform may be advantageously accomplished by use of the perforator (56).

**216. The more recently published method of G. Savini\*** is on the same lines, with advantageous modifications: Heat 12 gm. of the sample for 10 minutes with 70 mls of petroleum benzin, decant and filter the benzin; repeat the extraction with two fresh portions of benzin. Transfer the filter to the flask containing the residue of the cacao and boil with 250 mls of water and 5 mls of diluted sulphuric acid (10%) for one hour under a reflux condenser. Cool the solution to 30° C. and make up with water to 300 mls. Filter, mix 250 mls of the filtrate (= 10 gm. of the sample) with 10 gm. of sand and excess of magnesium oxide, evaporate to a syrup and mix with it 8-10 gm. of magnesium oxide to produce a dry powder. Boil this in a flask 15 minutes under a reflux condenser with 100 mls of chloroform and 0.25 ml of strong water of ammonia. Repeat the extraction with chloroform until 500 mls altogether have been used. Evaporate off the chloroform, wash the residue twice with petroleum benzin (5 mls), which is passed through a filter and rejected. The residue is boiled repeatedly with small portions of water, which is passed through the same filter, the solution is evaporated and the residue of caffeine and theobromine is dried at 80° C. to constant weight and weighed.

**217. Theobromine and caffeine** are separately determined by the method of W. E. Kunze† as follows: Boil 10 gm. of the sample 20 minutes with 150 mls of 5 percent sulphuric acid, filter and exhaust the residue with boiling water. Add to the warm solution a large excess of phosphomolybdic acid; after 24 hours collect the precipitate and wash with about a liter of 5 percent sulphuric acid. Transfer the filter containing the precipitate to a beaker and add a solution of barium hydroxide in excess, then precipitate the excess of baryta with carbon dioxide. The whole is then dried on the water bath and extracted with boiling chloroform, the solvent is distilled

\*Ann. Chim. Appl., 1916, 6, 247.

†Zeitsch. Anal. Chem., 33, 1-29.



off and the alkaloids are left as a perfectly white residue, which is dried and weighed.

218. The mixed alkaloids are then dissolved in ammonia, the solution is heated to boiling and a considerable excess of silver nitrate (about 1.3 parts of silver to one of theobromine) is added, and the boiling continued until ammonia is all driven off and the liquid is reduced to a few mils. The caffeine remains in solution while the theobromine is precipitated as a silver compound. Caffeine is extracted from the solution by chloroform and determined in the usual manner (220), (224) and (225) while theobromine is estimated by igniting the silver precipitate, each gm. of residual metallic silver corresponding with 1.667 gm. of theobromine.

## CAFFEINE-CONTAINING DRUGS

219. **Caffeine differs from most other alkaloids** in the following particulars, (1) it is quite freely soluble in warm water, (2) it is but feebly basic, so that it cannot be determined by alkalimetric titration, (3) it is readily extracted from acid solutions by shaking out with chloroform, (4) it is not precipitated, even from strongly acid solutions, by Mayer's reagent, although a characteristic crystalline precipitate is produced by a solution of potassium iodide saturated with mercuric oxide. In an acid solution it is precipitated by Wagner's reagent, and it is also precipitated by tannin. It crystallizes easily from its solutions in volatile solvents, and may be easily sublimed at 180° C. without alteration.

220. **The assay of drugs containing caffeine** is generally a simple matter. Either hot water or some immiscible liquid is used for the primary solvent. In the former case it is generally expedient to add to the aqueous solution some lead subacetate, make up to a definite volume with water, filter, to the filtrate add (dry) sodium phosphate or other precipitant for the excess of lead, filter once more and shake out the alkaloid with chloroform from an aliquot portion of the solution. The alkaloid so obtained is often quite

white and almost free from impurities. It is to be weighed and then if it prove to be readily and completely soluble in warm water, it may generally be accepted as pure caffeine. When chloroform or carbon tetrachloride has been used as the primary solvent, it is expedient, if the drug contains fatty or waxy matters or chlorophyll, to add to the solution some solid paraffin before evaporating, extract the caffeine from the residue with hot water, and finally recover it in relatively pure form by shaking out with chloroform. Complete extraction with chloroform is facilitated by adding to the solution potassium nitrate.

**221. In either case,** the caffeine should be quite white and well crystallized. It should dissolve completely in moderately hot water ( $60^{\circ}\text{C.}$ ). Impurities may be removed by heating the alkaloid (less than 0.5 gm.) with 2 mls of strong sulphuric acid in a water bath 10 minutes, diluting cautiously with water, rendering alkaline with ammonia and shaking out with chloroform. (In all cases make sure that the whole of the alkaloid is extracted. After shaking out four times, repeat the operation with 10 mls more of the solvent, evaporate one mil of the fluid to dryness, treat the residue with a few drops of diluted hydrochloric acid and one drop of Wagner's reagent; no precipitate should be produced.)

**222. The crude caffeine may be sublimed** by a regulated heat, when most impurities will be left behind; with a suitable apparatus, the sublimate may be obtained in condition for weighing, with no appreciable loss. Purification of the crude alkaloid from traces of fatty matter may be effected by treatment with several portions of petroleum benzin, or by dissolving in hot water and adding a little solid paraffin, heating on the water bath a few minutes, cooling, removing the congealed paraffin (treating this if necessary with an additional portion of hot water)—the alkaloid being finally recovered by shaking out the aqueous solution with chloroform. When benzin is used, filter the successive portions through the same dry filter, which is to be warmed to dissipate the last of the benzin. Treat the dried filter with hot

water (20 mls) to dissolve traces of caffeine. Extract this when cool with chloroform, which is to be then used to dissolve the residual caffeine. Filter the chloroformic solution if necessary, evaporate, dry to constant weight at 80° C. and weigh.

**222½.** In case theobromine is present as an impurity, extract the alkaloidal residue with carbon tetrachloride, which dissolves only the caffeine. See (217) and (218).

**223. Partial purification** of the crude caffeine may be effected by dissolving it in 10 mls of 10 per cent hydrochloric acid and 50 mls of hot water. The solution is cooled, 50 mls of an iodine solution (iodine 10 gm., potassium iodide 20 gm., water 100 mls) is added and the mixture is set by over night. The precipitate is decomposed by sodium sulphite (or acid sulphite) and diluted sulphuric acid, the solution is made alkaline with water of ammonia and the caffeine shaken out with chloroform.

**224. Iodometric determination.** See (75). Moses Gomberg\* has shown that caffeine can be determined with a fair degree of precision by a volumetric process, involving its conversion into a periodide. His procedure, applicable only in absence of other substances precipitated by iodine, is as follows: To 25 mls of tenth-normal iodine solution, add a solution of the caffeine (not to exceed 0.025 gm.) in water acidulated with hydrochloric acid. Make up the volume with water to exactly 50 mls. Shake the mixture well and let stand until the precipitate separates so as to leave the supernatant fluid perfectly clear. The solution must be of a deep garnet color; if the color is pale a new experiment must be made using a smaller quantity of caffeine. Decant the clear solution through a pledget of asbestos fiber which has been previously soaked in an iodine solution, washed well with water and dried. Titrate 25 mls of this with tenth-normal sodium thiosulphate, deduct twice the volume in mls of the thiosulphate consumed from 25 and multiply the remainder by 4.853 to find

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\*Journ. Am. Chem. Soc., 1896, 18, 331-42.

the weight in milligrams of the anhydrous caffeine present in the sample taken.

225. The most exact determination of caffeine in residues of doubtful purity is made by a **nitrogen determination** by Kjeldahl's or Gunning's method. Multiply amount of nitrogen by 3.464 for weight of caffeine.

226. **Glucosidal Caffeine.** It has been repeatedly pointed out that even large quantities of boiling water fail to extract from some samples of tea the whole of the caffeine present. Prolonged boiling does finally take it all out. The same thing is observed in the case of some other plants which contain caffeine, notably kola. The compound, which some have regarded as of a glucosidal nature, but which has been shown to be in fact caffeine tannate,\* is broken up by boiling under reflux condenser half an hour or longer with highly dilute sulphuric acid (5 percent). It is advisable to adopt this as part of the routine of caffeine determinations when water is employed as the primarily solvent.

227. **Another point** to be kept in mind is that when caffeine is extracted from a drug by chloroform or carbon tetrachloride in a soxhlet apparatus, some moisture must be present to secure complete exhaustion of the drug.

228. Detail of assay proposed tentatively by A. O. A. C. ("**modified Stahlschmidt method**")† Put into a 500 mil flask 3.125 gm. of the sample in No. 40 powder, add 225 mls of water and boil 3 hours under a reflux condenser. Add 2 gm. of dry basic lead acetate (Dr. Horn's) boil 10 minutes, cool to room temperature, transfer to a 250 mil measuring flask and make up to 250 mls with water. Mix thoroughly, filter through a dry filter, remove lead from 200 mls of the filtrate with hydrogen sulphide, make up to 250 mls, filter through a dry filter and measure of the filtrate 200 mls as the equivalent of

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\*See paper by J. W. T. Knox and A. B. Prescott in Proc. Am. Pharm. Assoc., 1897, 131-67.

†For original Stahlschmidt method, see Chem. Centralblat, 1861, 396.

2 gm. of the sample. Concentrate this on a water bath to 40 mls, transfer (with rinsings) to a separator and shake out with chloroform, 25, 20, 15 and 10 mls. (When coffee is assayed, add a little ammonia before extracting the alkaloid.) Evaporate the chloroform, dry the residue (which should be crystalline and quite white) at 80° C. to constant weight and weigh. Purity of the caffeine to be established by determination of nitrogen. (The method was intended for assay of tea. It is said to serve well also for coffee. In any case ascertain whether the caffeine is completely soluble in warm water. If not, filter the aqueous solution and shake out again with chloroform. The pharmacist will prefer to use sodium phosphate (dry powder) rather than hydrogen sulphide to remove the excess of lead).

**229. Alternative method**, tentative, of A. O. A. C. ("Gorter method, modified"). Moisten 10 gm. of finely powdered coffee with 3 mls of water, allow to stand 30 minutes and extract with chloroform 3 hours in a soxhlet. Add 1 gm. of paraffin and evaporate the chloroform. Treat the residue repeatedly with hot water to extract the caffeine (222). Shake out the filtered aqueous solution with chloroform (at least four portions). (Wash the successive portions of chloroform with a strong solution of sodium carbonate and then with water, before evaporating.) Dry the residue of caffeine and weigh, then determine nitrogen in it by Kjeldahl or Gunning method and multiply by the factor 3.464 to find pure caffeine in 10 gm. of the sample. [Instead of using the paraffin, the initial chloroform solution may be evaporated and the residue treated with several successive portions of petroleum benzin to remove fatty and waxy matter. See (222).]

**230. An approved assay process** for coffee is that of Fendler and Stueber.\* Put into a glass stoppered bottle 10 gm. of coffee in moderately fine powder with 200 mls of chloroform and 10 mls of water of ammonia (10%) and shake continuously half an hour. Evaporate 150 mls of the chloroform, driving off the

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\*Berlin Zeitschr. Nahr-Genussm., 28, 9-20.

last of the solvent by a current of air. Digest the residue on a water bath 10 minutes with 80 mls of hot water, with frequent shaking. To the solution when cool add for raw coffee 10 mls, for roasted coffee 20 mls, of a 10 percent solution of potassium permanganate and let stand 15 minutes. Remove excess of the permanganate by gradual addition of just sufficient hydrogen dioxide, heat 15 minutes on a water bath, cool and filter through a moistened filter. Shake out combined filtrate and washings with chloroform (50, 25, 25 and 25 mls). Evaporate off solvent, dry residue and weigh as caffeine.

**231. Simplified assay process** adapted to ordinary requirements of the pharmacist. Digest 12 gm. of the powdered sample (in case of guarana, 6 insufficient) with 120 mls of chloroform, and 10 mls of water of ammonia (10 percent) with frequent shaking during one hour. Let stand 3 hours, decant the chloroform rapidly through absorbent cotton, avoiding evaporation. Measure out 100 mls of this, representing 10 gm. of the sample (5 in case of guarana); if not perfectly clear, the solution is to be filtered and the filter washed twice with fresh chloroform; unite the chloroform solutions in a beaker, evaporate to dryness (using air current at the end), treat the residue repeatedly with water (20 mls) at 80° C. to extract caffeine. Add to first portion 0.5 gm. of magnesium carbonate and when cool filter through a small plain filter, through which are to be filtered in succession, when cold, the remaining portions. Shake out the collected filtrates to which have been added a few drops of water of ammonia, with four portions (or more if necessary) of chloroform (25, 25, 20 and 15 mls). Evaporate off chloroform with due caution, dry the residue to constant weight at a temperature not above 80° C. and weigh.

**232. Alternative simplified assay.** Treat 10 gm. of the powdered sample with 30 mls of hot water, macerate 15 minutes and evaporate on a water bath until the powder is barely moist. (In case of guarana, use 5 gm. and do not allow temperature to rise above 50° C.) Exhaust the powder in a small percolator

with chloroform, continuing the percolation until a few drops of the percolate, evaporated and taken up with water, show no cloudiness on adding tannin solution. Continue the assay as in the foregoing paragraph, beginning at "evaporate to dryness," line 11.

233. **Fluidextracts or tinctures** are to be evaporated to drive off the greater part of the alcohol. Add hot water (120 to 150 mls), digest 15 minutes on a water bath, add enough solution of lead subacetate to give the mixture a distinct sweet taste, cool and make up with water to 200 mls. Filter through a dry filter, add dry sodium phosphate sufficient to precipitate the excess of lead, let stand half an hour, then filter once more through a dry filter. Take 150 mls of the filtrate for extraction of the alkaloid by shaking out with chloroform. Complete the assay as in (231). The quantity of fluid taken for the assay should be equivalent to about  $13\frac{1}{2}$  gm. of tea or coffee, 16 gm. of kola or maté or 8 gm. of guarana, the aliquot taken being three fourths of these quantities.

234. Kola contains **theobromine** as well as caffeine. The two alkaloids, according to G. Goris, may be determined in the following manner.\* Mix 15 gm. of powdered kola with 10 gm. of calcined magnesia, moisten with 40 mls of water and dry at  $25^{\circ}$ — $30^{\circ}$ C. Powder once more and exhaust in a soxhlet with 150 mls of carbon tetrachloride, which extracts caffeine but not theobromine. This will require 10 to 12 hours, Evaporate the solvent, dry and weigh the alkaloid, which is practically pure caffeine. Extract the residue in the soxhlet with chloroform to obtain the theobromine. Dohme and Engelhardt† make no separate determination of theobromine. They direct to boil the powdered kola with a mixture of alcohol one volume, water two volumes, 3 hours under a reflux condenser. The solution is then filtered, evaporated nearly to dryness on a water bath, mixed with calcined magnesia and sand, dried and extracted by boiling

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\*Repertoire, 1914, 26, 171.

†Proc. A. Ph. A., 1896, p. 599.

with chloroform. Charles\* directs to mix 10 gm. of the finely powdered drug with 1 gm. of slaked lime (magnesia would surely be preferable, since it has been shown that lime acts on caffeine) and 20 gm. of 80 percent alcohol, evaporate on a water bath to 14 gm., exhaust by boiling with four successive portions (35, 35, 30 and 25 gm.) of a mixture of alcohol 20 gm., chloroform 100 gm. Evaporate, and extract the residue with slightly acidulated boiling water.

**235. Caffeine Sodio-benzoate.** Caffeine is easily determined in its combinations with alkali benzoates and salicylates (remarkable for their ready solubility in water) by dissolving 1 gm. in 5 mils of distilled water, adding 5 mils of sodium hydroxide solution (5 percent) and shaking out the caffeine with successive portions of chloroform (20, 10, 10 and 5 mils). The chloroform is evaporated off, the residue dried to constant weight at 80° C. and weighed. The U. S. P. requires that the salt after drying to constant weight at 80° C. shall yield not less than 46 nor more than 50 percent of anhydrous caffeine. Benzoic acid may be determined in the solution from which caffeine has been extracted, by making it acid with diluted sulphuric acid and shaking out with chloroform (606). Each gm. of benzoic acid corresponds to 1.180 gm. of sodium benzoate ( $\text{Na C}_7\text{H}_5\text{O}_2$ ), the official salt however being permitted to carry 1 percent of impurities of a nature not specified, in addition to possible hygroscopic moisture.

**236. The U. S. P. requirement** for caffeine sodio-benzoate is a minimum of 46 percent and a maximum of 50 percent of anhydrous caffeine in the dried salt. This range is far too wide. The product is supplied by manufacturers who assume that it is to be made by combining equal parts by weight of caffeine and sodium benzoate. The caffeine they take to be the monohydrated crystals, carrying nearly 8.5 percent of water of crystallization,† while the

\*Annal. de. Chim. Anal., 1, 345: Proc. A. Ph. A. 1897, p. 533.

†Caffeine crystals in fact effloresce rapidly, retaining not more than one third of a molecule of  $\text{H}_2\text{O}$ .



sodium benzoate must conform to the U. S. P. rubric of purity (99 percent). The product should therefore show not far from 47.7 percent of anhydrous caffeine, so that a reasonable requirement would be a minimum of 47.5 percent and a maximum of 48 percent of anhydrous caffeine. (Merck's product, it may be noted, is stated to contain 47.9 percent. As a matter of fact, crystallized caffeine soon loses the greater part of its water of crystallization, so that when equal weights of caffeine taken from stock and sodium benzoate (99 percent) are used, the product will contain more nearly 50 percent of anhydrous caffeine. The careful manufacturer, however, will surely make due allowance for loss in weight by efflorescence of the caffeine he uses.)

237. **Caffeine sodio-salicylate** is official in the German Pharmacopoeia and has found a place in the National Formulary. According to the latter authority it is made by combining equal parts of caffeine and sodium salicylate. Assuming that the caffeine contains one molecule of water of crystallization, the product should contain when thoroughly dried 47.78 percent of anhydrous caffeine. As in the case of caffeine sodio-benzoate, a reasonable requirement would be not less than 47.5 nor more than 48 percent of anhydrous caffeine. The Swiss Pharmacopoeia makes the requirement 44 to 46 percent anhydrous caffeine, "corresponding with 48 to 50 percent of caffeine," but these figures do not gibe. The German Pharmacopoeia combines caffeine and sodium salicylate in the proportion of 5 : 6. Assuming that the caffeine contains 1 molecule of water of crystallization, the product when completely dry should contain 43.27 percent of the anhydrous alkaloid (the text makes it 43.8 percent). The requirement, however, is of not less than 40 percent, the product being permitted to carry only 5 percent of water. Again, the quantity of chloroform used to extract the alkaloid is stingy, and the alkaloid is dried at 100° C., involving certainly risk of some loss by volatilization.

## CINCHONA

238. **The following facts** are to be kept in mind in assaying cinchona bark: (1) Chloroform reacts slowly with alkaloids, including those of cinchona, with formation of hydrochlorides of the bases, hence chloroformic solutions of these alkaloids must not be allowed to stand any length of time before distilling or evaporating off the chloroform (unless in contact with ammoniated water). (2) When the cinchona alkaloids are extracted from an ethereal or chloroformic solution by shaking out with an acid, the solution will contain impurities which should be removed if a gravimetric determination is to be attempted. This may be done by rendering the solution as nearly as possible neutral by cautious addition of weak alkali. A bit of litmus paper may be used as an indicator, the solution being brought just to the verge of alkalinity. The precipitate produced is to be filtered out, and the filter washed with water, the washings added to the neutral solution, from which the alkaloid is then to be extracted by shaking out in the usual manner. (3) In titration of cinchona alkaloids, the only indicators among those in common use, which give satisfactory results are methyl red and haematoxylin—the latter apt to be erratic in the color changes it produces.\* However, titration of the alkaloids is on the whole preferable to weighing them.

239. **The titration factor** for total alkaloids of cinchona may be taken as 0.0309 for tenth-normal acid. It is noticeable that while with most indicators quinine sulphate is a neutral salt, with methyl orange the bisulphate shows as neutral, although the "end point" of the titration is not sharp. The same peculiarity is not shown by cinchonidine. For titration of

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\*E. Rupp and K. Seegers (Apoth. Ztg. 1907, 748-50) recommend as satisfactory indicators in titration of cinchona alkaloids, certain derivatives of phenolphthalein, viz. dinitrophenolphthalein, p-nitrophenol and tetrachlor-tetrabrom-phenolphthalein, the last named particularly useful when the solution to be titrated is colored.

quinine, azolitmin or the official solution of litmus may be used, although not as satisfactory as methyl red or haematoxylin. The other ordinary indicators are useless. For cinchonidine, uranin as well as the foregoing, is available, while lacmoid and cochineal, pushed to full color change in residual titration, give fairly good results. Other indicators are unsatisfactory with exception of iodeosin, which may be used for total alkaloids by one having exhaustless patience.

**240. The method of assay known as Fromme's** is now generally regarded as superior to all others in convenience and effectiveness. As modified by Friedrichs and Mannheim,\* this is carried out as follows: Heat in a flask 2.5 gm. of powdered bark (not necessarily in fine powder) with a mixture of 2 mls of strong hydrochloric acid and 20 mls of water, 10 minutes on a water bath†. After standing one hour add 100 mls of a previously cooled mixture of ether and chloroform in the proportion (by volume) of 1 to 3.5, together with 5 gm. of sodium hydroxide, and shake the mixture vigorously two minutes. Add then 1.5 gm. of powdered tragacanth to clarify the solution, and decant 80 mls of this, representing 2 gm. of drug. Add 15 mls of alcohol and evaporate nearly to dryness, then add 10 mls of ether and 30 mls of water with a few drops of haematoxylin indicator and determine the alkaloids by direct titration with tenth-normal hydrochloric acid, shaking after each addition, to the production of a lemon yellow color. Each mil of the volumetric acid corresponds with 0.0309 gm. of cinchona alkaloids.

**241.** According to Fromme, the alkaloids should preferably be determined gravimetrically, being shaken out first from the ether-chloroform solution with 1 percent hydrochloric acid (15, 10 and 10 mls, or more if necessary) then from the aqueous solution made alkaline with ammonia, with chloroform (15,

\*Arch. Pharm., 1915, 130; cf. Pharm. Ztg., 1905, 770.

†W. L. Scoville dries the bark after treating it with hydrochloric acid, finding that when this is done the crude alkaloid extracted is pure enough for gravimetric determination.

10 and 10 mils), the successive chloroform solutions being filtered through a double filter, the chloroform evaporated off and the residue dried to constant weight at 100° C. and weighed. The residue should be taken up with alcohol (5 mils), dried once more and brought to constant weight at 100°—115° C. See (238) and (243). [The alkaloid extracted in this manner will surely be far from pure (Ed).]

**242. Another titration method** of determining total alkaloids of cinchona bark is to evaporate to dryness an ether-chloroform solution representing 2 gm. of the bark, dissolve the residue in 50 mils. of twentieth-normal hydrochloric acid and add 50 mils of twentieth-normal solution of picric acid (11.45 gm. pure picric acid in 1 liter). Shake thoroughly until the liquid has become clear and the alkaloid picrate is completely aggregated, and filter. Reject the first portion of the filtrate, then collect 50 mils and mix with 50 mils of alcohol. Titrate the excess of acid with tenth-normal alkali and phenolphthalein. The change from yellow to red denotes the end point. Subtract the number of mils of tenth-normal alkali from 20 and multiply the remainder by 0.0309 to find the quantity in grammes of alkaloid present. For a sharper end point, add 10 mils of a neutral solution of 4 gm. of potassium iodide and 1 gm. of potassium iodate in 100 mils of water, stopper securely and allow to stand 10 minutes, then titrate the liberated iodine with tenth-normal sodium thiosulphate solution. See (75).

**243. Approximate method of Florence.\*** Place in a flask 12 gm. of the finely powdered sample, add 175 mils of alcohol-free ether and 10 mils of a 10 percent solution of sodium hydroxide. Stopper the flask and shake frequently during 1 hour. Add 10 mils of water, shake and decant the ether into a 250 mil separator, avoiding evaporation; add 25 mils of lime water, shake, draw off the lime water and reject it. Transfer 100 mils of the ether to a wide-necked stoppered flask, add 30 mils of water and run in from a burette tenth-normal ethereal solution of oxalic

\*Bull. Sci. Pharm., 13, 365-8; Chem. Zentr., 1907, I, 130-1.

acid (0.63 gm. crystallized oxalic acid in ether sufficient to make 100 mls) until the solution is exactly neutralized (to litmus). The alkaloids are thus all converted into oxalates, all but the quinine salt going into solution in the water. Each mil of the oxalic acid solution corresponds with 0.0309 gm. of total cinchona alkaloids. Collect the precipitate of quinine oxalate on a tared filter, wash it with a little water, dry and weigh. Each gramme of the salt corresponds with 0.878 gm. of anhydrous quinine.

**244. Assay by acid** is still practised by some, and gives fairly satisfactory results. According to Dr. De Vrij, mix 20 gm. of the bark in fine powder with 20 mls of water to which has been previously added 3 mls of strong hydrochloric acid. Let stand a few hours, then add more water, stirring thoroughly, to form a liquid which can be poured. Let stand until foam disappears, introduce into a cylindrical percolator provided with a loosely packed plug of cheese cloth, over which there is placed a layer of fine siliceous sand; percolate to exhaustion with water, about 180 to 200 mls generally sufficing. From the solution the alkaloid may be recovered (a) by precipitating with sodium hydroxide in large excess (solubility correction 0.585 mg. for each mil of fluid, or (b) by making alkaline with sodium hydroxide and shaking out at once with benzene (1000 and 200 mls).

**245. Assay of U. S. P. IX.** The general assay process of (102) is followed, using 5 gm. of drug in No. 40 powder, with 200 mls of ether-chloroform (2:1 vol.)\* with 8 to 10 hours digestion. The aliquot taken represents 4 gm. of drug. This is shaken out with acid, the solution made alkaline with ammonia and the alkaloid extracted by shaking out with chloroform. After evaporation of solvent, the residue is dissolved in alcohol and re-dried twice in succession to drive off all traces of chloroform, and finally dried at 100° C. to constant weight and weighed. The result leaves much to be desired in the way of exactness. The acid solution should be rendered almost

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\*The proportions should be either 3 : 1 (lighter than water) or 3 : 4 (heavier than water) preferably the former.

neutral to litmus, retaining faint acidity, when a precipitate is thrown down, not alkaloidal in character. This should certainly be eliminated before extracting the alkaloids for weighing. Titration of the alkaloids is much to be preferred, using haematoxylin or methyl red as indicator. The assay of U. S. P. IX is content with a determination simply of total alkaloids, making a single minimum standard of 5 percent for both yellow and red cinchona. In the U. S. P. VIII there was an added requirement in the case of the yellow bark, that it should contain 4 percent of anhydrous ether-soluble alkaloids.

**246. Method of German Pharmacopoeia** (said to yield very satisfactory results). Twelve gm. of the finely powdered drug are treated with 30 gm. each of chloroform and ether, and 5 gm. each of 15 percent sodium hydroxide solution and water, and shaken frequently during 3 hours; 60 gm. of ether are added and, after shaking, an aliquot (= 8 gm. of drug) is taken and distilled to one third. The alkaloid is shaken out with highly dilute (1 + 99) hydrochloric acid (20 and 5 mls); the solution is made alkaline with sodium carbonate and the alkaloid extracted by shaking out with chloroform (5, 5, 5 and 5 mls) and is then brought into aqueous solution by shaking out once more with 25 mls of tenth-normal acid followed by 3 portions of water (10 mls); water is added to make up 100 mls, and 50 mls of this (= 4 gm. drug) are finally titrated with tenth-normal potassium hydroxide solution using a freshly prepared solution of haematoxylin as indicator. The method is very circumstantial, and appears to be needlessly complicated. Results are said to agree well with those reached by the Fromme method.

**247. The British Pharmacopoeia** recognizes only red cinchona, and adheres to its traditional method of assay, viz. treating the powdered drug with slaked lime and a little water, drying the mixture, and exhausting by boiling repeatedly and finally percolating with a mixture of 3 volumes of benzene with 1 volume of amylic alcohol. The alkaloids are shaken out with diluted hydrochloric acid, the solu-

tion is carefully neutralized with ammonia and Rochelle salt is added, precipitating together the quinine and cinchonidine as tartrates. Eighty percent of the dried precipitate is reckoned as alkaloid (quinine and cinchonidine), while the other alkaloids are extracted from the mother liquor by adding ammonia and shaking out with chloroform.

**248. The method of the French Codex** follows also traditional lines which furnish no suggestions for improvement on the more modern processes. It includes a method of determining the quinine as well as the total alkaloids, but this also is antiquated.

**249. Assay by Ammonium Thiocyanate.** In presence of zinc sulphate, ammonium thiocyanate precipitates quantitatively the alkaloids of cinchona bark as pointed out by P. W. Robertson.\* It should be possible to determine the alkaloids in the crude mixture obtained from a sample of cinchona bark by dissolving in acid, neutralizing, adding zinc sulphate and a measured excess of volumetric ammonium thiosulphate, and titrating the excess of thiosulphate remaining in solution. The details of such an assay process have not been worked out, but the suggestion is worth considering.

**250. Titration of alkaloids in form of hydrochlorides.** Julius Katz† directs to dissolve the alkaloidal residue from 4 gm. of drug in alcohol, add 10 drops of hydrochloric acid and 0.25 gm. of sodium chloride and evaporate with stirring to complete dryness. Heat 15 minutes in the water oven, to dissipate the last traces of hydrochloric acid, dissolve in neutral alcohol, add 5 drops of a 0.2 percent solution of Poirrier's blue as indicator and titrate with tenth-normal alcoholic potassium hydroxide. Each mil of the volumetric solution corresponds with 0.0155 gm. of anhydrous cinchona alkaloids.

**251. Assay by Petroleum Oil** (kerosene) has sometimes been practised and is well adapted for

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\*i roceedings Chem. Soc., 1905, 242.

†ber. Deutsch. pharm. Ges., 1910, 20, 316-29.

operations on a large scale. E. Landrin\* directs to mix 30 gm. of finely powdered bark with 7.5 gm. of slaked lime suspended in water and 7.5 gm. of a 40 percent solution of sodium hydroxide, add 200 mls of kerosene and heat on the water bath 20 minutes with frequent shaking. Decant the solvent and repeat the operation with a fresh portion (200 mls) of kerosene. Shake out the alkaloids from the mixed kerosene solutions with several successive portions of five percent sulphuric acid (20, 20, 15, 10 mls. The assay may be carried on from this point by any method the operator may choose.

**252. Method of P. J. Kruysse†** (for determination primarily of quinine): Moisten 6 gm. of coarsely powdered cinchona with 1.5 mls of water of ammonia and 1.5 mls of water, add 6 gm. of sand and triturate 15 minutes in a mortar, thus reducing the cinchona to a fine powder. Add 3 gm. of slaked lime and 3 mls of water, mix well, transfer to a flask by aid of acetone, of which 150 mls are used altogether. Boil half an hour under a reflux condenser; when cool, decant the acetone and rinse the flask and its contents with several portions of acetone, bringing the total volume thus to 180 mls. From 150 mls of this distil off the solvent, dry the residue on the water bath, add 1.5 mls of hydrochloric acid and 20 mls of water to dissolve the alkaloids. Filter the solution and wash the filter with several portions of water acidulated with hydrochloric acid. Remove fatty matters by shaking with ether or chloroform, treating this subsequently with acidulated water to recover any alkaloid which may have been taken up. (This treatment for removal of fat is imperative in the assay of red cinchona; it may be omitted in the case of Ledgeriana bark.) Warm the united acid solutions and neutralize exactly, using litmus as indicator, heat to boiling and add 0.5 gm. of ammonium oxalate. Cool the solution, when in case of a bark rich in quinine crystals of quinine

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\*Compt. Rend., 108, p. 750; Rep. de Pharm., 1889, 212.

†Chem. and Drugg., 1915, 155. See also Pharm. Merkblad, 1912, No. 49.



oxalate are at once deposited. If crystals do not form within ten minutes, heat the solution again to boiling and dissolve in it 227 mg. of crystallized quinine hydrochloride, corresponding with 0.5 gm. of quinine sulphate. Cool the solution and after half an hour collect the crystals of quinine oxalate on a filter, wash them with 2 portions of distilled water (10 mls), dry at 100° C. and weigh. To find the quantity of quinine, estimated as crystallized quinine sulphate, in 5 gm. of the bark, multiply the weight of the crystals by 1.2 [more exactly 1.182 (Ed.)] and add 0.020 gm. for water solubility of quinine oxalate. If it has been necessary to add the quinine hydrochloride (above) deduct 0.500 gm.

253. **In case of red bark**, the quinine oxalate is contaminated with much cinchonidine oxalate; so that it is necessary to purify it by the following procedure: Dissolve the salt in 75 mls of boiling distilled water, add 0.5 gm. of sodium nitroprusside, cool the solution, collect the crystals of quinine nitroprusside, dry and weigh. Multiply the weight by 1.04 to convert to crystallized quinine sulphate and add 0.040 gm. as solubility correction (deduct if necessary 0.500 gm. for added quinine hydrochloride). Cinchonidine can be determined by precipitating from the combined filtrates with Rochelle salt as cinchonidine tartrate. Add for each ml of the solution 0.00085 gm. as solubility correction and multiply by 0.804 for anhydrous cinchonidine.

254. **Assay of galenical preparations of cinchona.** For a fluidextract employ type process A (110), using potassium hydroxide in preference to ammonia in the primary extraction, haematoxylin being given preference as indicator. Alternative methods for determining total alkaloid are those of (242) and (243).

255. **Fairly good results** may be obtained by adding to 4 mls of the fluidextract 85 mls of alcohol followed by 1.5 ml of solution ferric chloride (U. S. P.) then by 5 mls of a 10 percent solution of sodium hydroxide, shaking vigorously a few minutes and mak-



paper, and note the quantity of acid used as a guide in future operations. Evaporate off alcohol and dissolve the residue in water at  $85^{\circ}\text{C}$ . using 5 mils of water for each mil of volumetric acid above. If necessary add a few drops of volumetric acid to effect complete solution. Maintaining the temperature at  $85^{\circ}\text{C}$ ., add cautiously, drop by drop, tenth-normal sodium hydroxide until all but neutral.

260. Cool the solution rapidly to  $15^{\circ}\text{C}$ ., keep it at that temperature one hour, collect the crystals on a pair of counterpoised filters and wash with a little cold water (1.5 mil for each mil of volumetric acid used in the preliminary titration). Drain the crystals, press, dry at  $100^{\circ}\text{C}$ . and weigh (in a securely stoppered weighing bottle, since the salt is very hygroscopic) as anhydrous quinine sulphate. Measure the filtrate and washings, and for each mil add 0.00817 gm. for solubility of quinine sulphate in water. Multiply by 0.8686 for quinine alkaloid. [If very accurate results are desired, determine the quinine by converting it into herapathite. See (265) and (268) Ed.]

261. To portion B add dilute hydrochloric acid to faint acid reaction, evaporate to dryness and dissolve the residue in a minimum quantity of water at  $38^{\circ}\text{C}$ . Neutralize accurately with tenth-normal sodium hydroxide, add in excess a saturated solution of sodium and potassium tartrate, cool and keep at  $15^{\circ}\text{C}$ . one hour with frequent stirring. Collect the crystals of cinchonidine and quinine tartrates on a pair of counterpoised filters, wash with 100 mils of water at  $15^{\circ}\text{C}$ ., dry at  $104^{\circ}\text{C}$ ., and weigh. Add for each mil of filtrate and washings 0.00083 gm. to find weight of combined tartrates of cinchonidine and quinine; deduct the weight of quinine tartrate, found by multiplying the weight of anhydrous quinine sulphate (260) by 0.915. The remainder, multiplied by 0.90, gives anhydrous cinchonidine. (This result may be checked by precipitating from the residual solution including washings of (260), after proper titration, the cinchonidine tartrate precisely as above, deducting from the weight an amount corres-

ponding with the correction for solubility of quinine sulphate and multiplying the remainder by 0.804.)

262. **Concentrate the filtrate** and washings from the tartrate to its original volume, cool, render faintly acid with acetic acid and add with constant stirring an excess of a neutral saturated solution of potassium iodide. After an hour or so collect, wash, dry and weigh the **quinidine hydriodide** precisely as in the case of the tartrate. Add 0.00077 gm. for each mil of filtrate and washings and multiply by 0.7188 for **anhydrous quinidine**.

263. From the filtrate and washings precipitate **the rest of the alkaloid** with sodium hydroxide, collect on a pair of counterpoised filters, dry at 104° C. and weigh. Heat with 40 percent alcohol to dissolve out amorphous alkaloid, dry the residue at 104° C. and weigh as **anhydrous cinchonine**. The difference between this weighing and the previous one gives the quantity of **amorphous alkaloid**, subject to correction by deducting 0.00066 gm. for each mil of filtrate from the precipitated tartrates and 0.00052 gm. for each mil of filtrate from quinidine hydriodide.

## DETERMINATION OF QUININE

### IN ALKALOIDS FROM CINCHONA.

264. **I. Separation by sparing solubility of the sulphate.** See (260). The French Codex makes this method official. Vigneron\* improves it in the following manner: Treat the alkaloids from 25 gm. of bark with 20 times their weight of ether, add six "small pieces" of pumice soaked in alcohol and shake occasionally during 6 hours. Decant the ether through a small filter and treat the residue 12 hours with the same volume as before of fresh ether. Pass the ether through the same filter, which is to be washed once with ether. The mixed ethereal solutions are allowed to evaporate and the residue is dissolved in 5 mls of alcohol and 100 mls of water saturated at room temperature with quinine sulphate. Heat on a water bath to expel alcohol and ether, and at the same time add normal sulphuric acid exactly to the point of neutrality, using haematoxylin as indicator. When the solution is cold, collect the crystals of quinine sulphate, wash them with a saturated solution of quinine sulphate, dry and weigh. Test the purity of the salt by dissolving 0.5 gm. of it by aid of heat in 100 mls of water saturated in the cold with quinine chromate, and precipitating with a 5 percent solution of potassium neutral chromate. Multiply the weight of the dried chromate by 0.993 to find that of the corresponding crystallized quinine sulphate.

265. **II. Method of De Vrij,† by precipitation as herapathite.** Dissolve the mixed alkaloids in 40 times their weight of alcohol of 92 percent containing 0.76 percent of sulphuric acid (or dissolve in normal sulphuric acid, using 6.2 mls for each gramme of alkaloid, evaporate to one half and add 46 mls of strong alcohol for each gramme of alkaloid). If the bark under examination contain a large proportion of cinchonidine, digest the crude alkaloid in powder with ten times its weight of ether; after standing an hour or more decant the ether solution and wash the

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\*Journ. Pharm. Chim., 1905, 21, 180-3.

†Pharm. Journ. and Trans., 1875, p. 461; The L'ague, July 5 1880.

residue twice with a few mils of ether. Evaporate the united ether solutions, which will contain all the quinine, and employ the residue instead of the total alkaloids for the test.

266. Prepare a solution of chinoidine iodosulphate in the following manner: Heat on a water bath one part of chinoidine with two of benzene. Decant the clear solution and agitate with an excess of diluted sulphuric acid. To the acid solution add slowly with constant stirring a solution of iodine 1 part, potassium iodide 2 parts, in water 50 parts. On warming, the precipitate of chinoidine iodosulphate subsides, and is to be washed repeatedly with water by decantation. Digest one part of this precipitate with six parts of alcohol on a water bath. Cool, and decant the clear solution, evaporate, and dissolve the residue in five times its weight of cold alcohol.

267. To the solution in sulphuric acid and alcohol of (265) add from a pipette, drop by drop. the foregoing reagent\* as long as it throws down a precipitate. As soon as all the quinine is precipitated and the reagent is in slight excess, the solution acquires an intense yellow color. Cover the beaker and heat to boiling on the water bath. Cool, note the volume of fluid, filter, wash the precipitate on the filter with a saturated solution of herapathite in 92 percent alcohol. Dry the precipitate at 100° C. and weigh. Add to the weight found for each mil of fluid previous to filtration 0.0011 gm. as a correction for solubility of herapathite in alcohol, multiply the corrected weight by 0.55055 for anhydrous quinine, or by 0.7409 for crystallized quinine sulphate.

268. **III. Treatment with ether** saturated with the **other alkaloids.** A saturated solution is prepared of cinchonidine, cinchonine and quinidine in ether containing 4 percent of alcohol. This is done in a room in which a constant temperature is maintained. A weighed quantity (about 0.5 gm.)

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\*In absence of this special reagent, use Christensen's iodine solution, prepared by dissolving 10 gm. of iodine in 10 gm. of 50 percent hydriodic acid and adding 8 gm. of sulphuric acid and 50 gm. of 70 percent alcohol.

of the mixed alkaloids is placed in a stoppered flask with 50 mls of the saturated ether and the mixture is shaken frequently during one hour. Twenty-five mls of the clear ether solution are transferred to a tared beaker, the ether is evaporated off and the residue is dried at  $125^{\circ}$  to constant weight and weighed. The weight minus the known weight of the alkaloids contained in 25 mls of the saturated ether at the temperature of the experiment is the weight of the anhydrous quinine contained in one half the sample taken. It is convenient to have a table (269) giving the weight of the dissolved alkaloids at different temperatures in 25 mls of the saturated ether. It may be deduced from the expression  $0.3857 + 0.00975(t - 14^{\circ}) + 0.0000625(t - 14^{\circ})^2$ , in which  $t$  is the temperature C. at which the test is made. The method is said to give good results, the error appreciable only when very little cinchonidine and cinchonine are present. It is the only method which gives good results when the amount of quinine is less than 20-30 percent of the total alkaloids.

269. **Weight of cinchona alkaloids other than quinine** dissolved at different temperatures by 25 mls of ether containing 4 percent of alcohol.

Temperature	Alkaloids dissolved	Temperature	Alkaloids dissolved
10° C.	0.3457	21° C.	0.4569
11	0.3559	22	0.4675
12	0.3660	23	0.4783
13	0.3757	24	0.4892
14	0.3859	25	0.5002
15	0.3955	26	0.5113
16	0.4054	27	0.5226
17	0.4155	28	0.5340
18	0.4257	29	0.5455
19	0.4360	30	0.5571
20	0.4464	31	0.5388

270. **IV. The Oxalate Method** of Shimoyama is a good one. See (243). The quinine may also be separated as chromate with fairly good results, provided the solution from 1 gm. of alkaloids is diluted before precipitation to 200 mls.

271. V. **Sodium Nitroprusside** has been suggested as an efficient precipitating agent for quinine. Kruyse has taken advantage of this reaction in his method of determining quinine in red cinchona (252). A study of the applicability of this principle to the assay of commercial salts of quinine is a desideratum.

#### ASSAY OF QUININE SULPHATE

272. **Water of crystallization.** Altogether the most important determination to make in a sample of quinine sulphate is that of water of crystallization. As received from the manufacturer, the salt contains the greater part of the 7 molecules of water of crystallization (14.45 percent) to which it is entitled according to the U. S. P., which however permits 16.2 percent, corresponding with nearly 8 molecules. (The British Pharmacopœia gives  $7\frac{1}{2}$  molecules, the German and French 8 molecules. The commercial salt, uneffloresced, contains about 14.5 to 16 percent.) As found on the apothecary's shelf the salt contains ordinarily not more than 7 to 8 percent of water. Exposed to dry air it will lose all but 2 molecules of its water of crystallization, the effloresced salt retaining 4.6 percent of its weight. The pharmacopœia prescribes that this salt shall be kept in well closed containers, but in the case of a salt at once so bulky and so highly efflorescent, it will require more than ordinary precautions to keep the salt of official standard.

273. The remedy would seem to be **to make official an effloresced salt** which will lose on drying completely 7 percent of its weight. This could be kept without especial precautions against loss or gain of weight. The anhydrous salt is highly hygroscopic, so that neither this nor the fully air-dried salt can be recommended. Under present conditions the quinine sulphate dispensed, whether on a physician's prescription or in a pharmaceutical preparation, is likely to contain 8 to 10 percent more quinine than the prescription or formula contemplated. It is to be assumed of course that "quinine sulphate" is the official salt containing not more than 16.2% of



water. The pharmacist should keep in stock an effloresced salt, ascertaining by experiment the quantity of water it has retained, noting this on the label of his container, and noting also the quantity equivalent to 1 part of the official salt, which may be taken to contain 15.32 percent of combined water, i. e.  $7\frac{1}{2}$  molecules of  $H_2O$ , although the formula given in the U. S. P. calls for only 14.45 percent (7 molecules.)

**274. To determine water of crystallization,** dry 2 gm. of the quinine sulphate three hours in the water oven, or one hour at  $110^\circ C.$ , cool in a desiccator and weigh quickly. If the quinine sulphate is dried at  $50^\circ C.$  it retains just 2 molecules of water, or 4.6 percent of its weight, and when a regulated temperature can be depended upon, this plan of determining water may be adopted.

**275. Quantity of "Official" Quinine Sulphate** corresponding with 1 part of an effloresced salt.

The salt shows $H_2O$	One part equivalent to U. S. P. official salt	Factor
4%	1.109	0.902
5	1.098	0.911
6	1.088	0.919
7	1.078	0.928
8	1.068	0.936
9	1.058	0.945
10	1.048	0.954
11	1.039	0.963
12	1.029	0.972
13	1.020	0.981
14	1.011	0.989
15	1.003	0.998
16	0.994	1.006

**Example.** The effloresced quinine sulphate loses in drying 8 per cent. In a formula 350 grs. of quinine sulphate are called for. The equivalent quantity of the effloresced salt will be  $350 \times 0.936 = 327.5$  grs.

**276. Detection of Cinchonidine** and other cinchona alkaloids in a quinine salt. It is practically impossible for manufacturers to exclude wholly from quinine salts the other alkaloids of cinchona bark. The presence of a small proportion, even as much as five percent does not appreciably affect the therapeutic uses of such a salt, neither does it cheapen the

product sufficiently to constitute a fraud. No simple plan has been found for determining accurately the proportion of such impurities, although many have been proposed. That which is most widely accepted is Kerner's ammonia test and this may well continue to serve as a conventional requirement that is sufficiently exacting.

**277. The ammonia test of Kerner.** In its original form the test was made by dissolving quinine sulphate in a prescribed amount of boiling water, cooling the solution to 15° C., keeping it one hour at that temperature, then filtering the solution and adding an equal volume of water of ammonia, which should produce a clear solution, or at least, redissolve any precipitate momentarily produced. Its most approved form is that adopted by the U. S. P. IX, which should be closely followed in every detail, as follows: dry about 2.5 gm. of the sample, which must be strictly neutral or slightly alkaline to litmus, at 50° C. 2 hours, cool in a desiccator and weigh out rapidly 1.8 gm. of the dried salt. Transfer this to a 50 mil Erlenmeyer flask, add 20 mls of distilled water, immerse in a water bath at 65° C., maintaining that temperature half an hour, occasionally shaking the flask. Cool to 15° C., and maintain this temperature 2 hours, then strain the liquid through muslin and filter it. Transfer 5 mls of the filtrate to a test tube, immerse in water at 15°, add 7 mls of water of ammonia (containing not less than 10 percent nor more than 10.2 percent of  $\text{NH}_3$ ) having a temperature of 15° C. Close the mouth of the test tube with the thumb and mix the liquids by inverting the tube gently twice. A clear solution results. The quantity of ammonia prescribed makes the test less strict than that of European pharmacopœias. Six mls would be not unreasonable and this is the quantity prescribed in the British Pharmacopœia, although in this case 2.0 gm. instead of 1.8 gm. of the effloresced salt are taken for the test; the German pharmacopœia, using 2 gm. of the effloresced quinine sulphate, prescribes 4 mls only, making the test much more stringent than ours.

**278. Application of Kerner's test** to other quinine salts. Of all the quinine salts used medicinally, the sulphate is the most likely to contain other cinchona alkaloids. The U. S. Pharmacopœia, however, employs Kerner's test, *mutatis mutandis* for the bisulphate, the hydrobromide, the hydrochloride and dihydrochloride, the salicylate and the alkaloid itself. The test is, however, not identical in the cases of the hydrobromide and hydrochloride, in that the solution in these cases contains sodium sulphate, a compound known to influence greatly the solubility of quinine sulphate. (To a lesser extent, this is true also of the test as applied to quinine bisulphate.) In all the tests of the U. S. P. the **same quantity of alkaloid** is used. In the German pharmacopœia, the quantity of quinine used in the hydrochloride test is much greater than that in the sulphate test. The U. S. P. tests for hydrobromide and hydrochloride should be made in the same way as that for the dihydrochloride, using the quantities of the respective salts prescribed in the present official tests. In testing quinine bisulphate, P. Bignelli directs to mix the sample (2.52 gm.) of bisulphate in a mortar with 1.5 gm. of lead carbonate, triturate 10 minutes with 10 mls of distilled water, transfer to a small flask, rinsing the mortar with 10 mls of distilled water, used in several successive portions; proceed then exactly as in Kerner's test.

**279. Oxalate test of Schaefer.\*** Put into a small tared flask 2 gm. of quinine sulphate, add 55 mls of water, heat to boiling and boil 2 minutes, then add a solution of 0.6 gm. of crystallized potassium oxalate (neutral) in 5 mls of water, cool to 20° C., bring the weight of the contents of the flask to 62.5 gm., and maintain a constant temperature of 20° C. 30 minutes, shaking the flask occasionally. Filter, and to 10 mls of the filtrate add one drop of a solution of sodium hydroxide (15 percent). In case other alkaloids than quinine are present to the extent of one percent a precipitate is produced.

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\*Arch. Pharm., (3) 25, 64 and 1033.

**280. Chromate test of De Vrij,\*** as modified by De Koningh†: Dissolve 2 gm. of the quinine sulphate (uneffloresced crystals) in 80 mls of hot water, add 12 mls of a 5 percent solution of neutral potassium chromate, allow the solution to stand some hours to crystallize, filter, add to the filtrate 5 mls of a 10 percent solution of sodium hydroxide when an immediate precipitate will indicate the presence of cinchonidine, quinidine or hydroquinine. Filter, shake the filtrate twice with chloroform (10 and 5 mls) to remove traces of these alkaloids, add 2 gm. of ammonium chloride and shake out again with chloroform, which will remove cupreine if present. Cinchonine is not detected by this test, since its chromate is as little soluble as that of quinine, but this alkaloid is not likely to be present in quinine sulphate.

**281. The Bisulphate method** is carried out in the following manner: Dissolve 5 gm. of quinine sulphate (uneffloresced) in 12 mls of normal sulphuric acid by warming, and set the solution aside 6 hours in a cool place to crystallize. Filter, make the filtrate alkaline with ammonia and immediately shake (1 minute) with ether (16 mls). Transfer the ether to a small flask, cool to 15° C. or below, and shake continuously at that temperature 10 minutes. The greater part of the cinchonidine will crystallize out either at once or after standing over night in a cool place. The crystals may be collected, dried and weighed. They probably contain some quinine, which may perhaps be separated by converting the alkaloid into sulphate, dissolving in 10 mls of water and adding 0.1 gm. of sodium nitroprusside.

**282. Barium chloride test.** Dry about 1 gm. of the sample at 50° C. 2 hours, in a tared weighing bottle and when cool, weigh. Dissolve this in 20 mls of distilled water, add a few drops of diluted hydrochloric acid, heat to boiling and, for each gm. of the effloresced salt, add exactly 25.56 mls of tenth-

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\*Arch. Pharm., (3) 24 and 1073.

†Ned. Tydschr. Pharm., 1897, 97.

normal barium chloride. Boil a minute or two and filter, divide the filtrate into two parts and add to one 0.5 mls of tenth-normal barium chloride, to the other a few drops of diluted sulphuric acid. If the salt is pure, neither will give more than a slight turbidity. If much cinchonine is present, the barium chloride will produce a distinct precipitate.

**283. Purity of quinine hydrochloride.** A simple test is given in the Hungarian Pharmacopœia. Dissolve 0.397 gm. of the salt in 10 mls of water containing a few drops of nitric acid, add 10 mls of tenth-normal silver nitrate and filter. Divide the filtrate into two portions, to one add a few drops of diluted hydrochloric acid, to the other 0.5 ml of tenth-normal silver nitrate. In neither case should there be produced a precipitate, although some turbidity is admissible. [A pure salt which has lost water of crystallization will show a precipitate with the silver nitrate. The salt should be rendered anhydrous by drying at 100° C. one hour, and of the dried salt 0.361 gm. should be taken for the test. Ed.]

## COCA

284. **In addition to cocaine**, coca contains in varying proportions cinnamylcocaine, tropacocaine, truxilline and ecgonine, from some of which it is practicable to obtain an equivalent of cocaine. The ordinary assay process determines the total alkaloids, which may be taken as a fair indication of the commercial value of the drug. An assay which extracts only benzin-soluble alkaloids gives a better idea of the therapeutic activity of the sample. However, it must be borne in mind that cocaine is a very unstable compound, so that any determination of the quantity present in a sample, whether of the crude drug or of a galenical preparation, shows merely the present value of the sample, which is almost sure to undergo continuous deterioration. Hence standardization of preparations of coca is an attempt at an unattainable ideal.

285. **The assay process** to be preferred is that of (102) employing petroleum ether\* as the immiscible solvent, and using only one-fourth the usual quantity of ammonia in the initial step of the assay. It is important to bear in mind that the alkaloidal residue cannot be dried without loss at a temperature higher than 90° C. The drying is best completed by aid of an air blast at a temperature not above 80° C. If the solvent is petroleum ether it is well to take up the residue twice with a little ether and re-evaporate. In alkalimetric titration, each mil of tenth-normal acid corresponds with 0.030318 gm. of cocaine. The U. S. P. IX does not recognize coca, so that an official standard is lacking. The U. S. P. VIII provided a minimum standard of ether-soluble alkaloids of 0.5 percent. Other pharmacopœias have required as much as 0.7 percent, but few of them now state any requirement.

286. **Kerosene assay of Dr. Squibb.**† Moisten 100 gm. of the powdered leaves with 100 mls of a 7

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\*See footnote to (47).

†Ephemeris, June 1888, 1101-6.

percent solution of crystallized sodium carbonate, pack at once in a cylindrical percolator, and exhaust by percolation with water white kerosene of which about 700 mls will generally suffice. (Presumably light liquid petrolatum may be used in place of kerosene.) Shake out the percolate thrice successively with 30 mls of water containing two percent hydrochloric acid. Wash the united acid solutions with 30 mls of ether to remove fatty substances, add 20 mls of ether and 10 mls of a 25 percent solution of sodium carbonate (sufficient to supersaturate the acid), shake cautiously, permitting the carbon dioxide to escape, then shake out with the ether, repeating the operation with two additional portions of that solvent; evaporate the ether, dry the residue and weigh as crude alkaloid.

287. **In the crude alkaloid** cocaine may be determined approximately by the method of Grandval and Lajoux. Add to the alkaloid ten times its weight of water with just enough hydrobromic acid to form a neutral salt, heat the solution on a water bath and saturate it rapidly with potassium bromide. On cooling the cocaine will crystallize out in the form of a double bromide of potassium and cocaine. To remove other alkaloids, percolate the crystals with a saturated solution of potassium bromide, in a small funnel having its tube rather firmly plugged with absorbent cotton. Finally dissolve the crystals in hot water and treat with ammonia and ether to recover the purified cocaine. (In dealing with cocaine, remember always how easily it is decomposed by alkalies, and indeed by heat alone.)

288. **William Garsed\* determines cocaine** in the crude alkaloid by dissolving this in diluted sulphuric acid and treating the solution with potassium permanganate, by which the cinnamyl cocaine is oxidized. Cocaine and truxilline remain unoxidized. They are recovered from the solution by shaking out.

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\*Pharm. Journ., 1903, 789-91.

with ammonia and ether and are then hydrolyzed with alcoholic potassium hydroxide, the solution is neutralized, the alcohol evaporated off, the residue dissolved in water made acid with sulphuric acid and shaken out with ether. Benzoic and truxillic acids are thus extracted, representing respectively cocaine and truxilline. The benzoic is separated from the truxillic acid by its comparatively ready solubility in water and is then determined by acidimetric titration. Each gm. of benzoic acid corresponds with 0.4026 gm. of cocaine.

**289. To determine cinnamylcocaine,** Garsed would acidify the solution of the crude alkaloid with sulphuric acid and add an excess of a volumetric solution of bromine. Each molecule of cinnamic acid absorbs  $2\frac{1}{2}$  atoms of bromine. Potassium iodide is added, and the liberated iodine, corresponding in amount with the excess of bromine, is titrated.

**290. Determination of cocaine in tablets, etc.** An aqueous solution is made, from which the alkaloid can generally be easily shaken out with ether and ammonia. This may be simply dried (below  $90^{\circ}\text{C.}$ ) and weighed, or the cocaine may be precipitated with platinum chloride. Dissolve 0.2 gm. of the alkaloid in 15 mls of water and 5 mls of diluted hydrochloric acid (10 percent), add an excess of platinum chloride and 80 mls of alcohol. When the precipitate has completely subsided, collect it on a tared filter, dry and weigh, add 0.022 gm. for each 100 mls of filtrate for solubility of the precipitate. Each gramme of the cocaine-platinum chloride represents 0.4228 gm. of cocaine. This method of determining cocaine is particularly useful in testing the strength of sterilized solutions, which are notoriously subject to deterioration. It is not seriously interfered with by the presence of boric acid, but sodium chloride prevents the complete precipitation of the double salt.



**COLCHICUM**

291. **Colchicine, the active constituent** of colchicum seed or corm resembles caffeine in its rather free solubility in water and in the fact that it may be removed from **acid** solutions by shaking out with chloroform. It differs from that alkaloid in yielding a precipitate with Mayer's reagent, although only in strongly acid solutions.

292. **Simple assay process** for colchicum corm. Put into a stoppered bottle or flask 15 gm. of the sample in fine powder, add a mixture of 10 mls of solution of lead subacetate with 25 mls of distilled water, digest 20 minutes at 55° C., then add 265 mls of distilled water, warm to 55° C., and digest at that temperature three hours with occasional shaking. [Avoid gelatinizing starch by too high temperature]. Cool, filter, add to the filtrate 0.75 gm. or more if necessary, of dry sodium phosphate, to precipitate excess of lead; after 15 minutes filter once more. Extract the alkaloid from 200 mls of the filtrate by shaking out repeatedly with chloroform (20 mls) until Wagner's reagent gives no turbidity when added to an acid aqueous solution of the residue from one ml of the chloroform. Distil or evaporate off the chloroform, dissolve the residue in a little alcohol and evaporate to dryness. Repeat this operation once more, then dry the residue to constant weight and weigh. Test the purity of the alkaloid by treating it with 20 mls of highly dilute sulphuric acid (0.25 percent) ten minutes at 70° C. If any residue remains undissolved, collect this on a pledget of absorbent cotton, wash it with 15 mls of water, with which the beaker or flask has been rinsed, press out carefully the water from the cotton, treat this with 5 or 6 mls of alcohol slowly added, then with a similar quantity of ether, making sure that all the residue is dissolved from the cotton. Evaporate the alcohol and ether, dry the residue and weigh. Subtract the weight from that of the crude alkaloid to find the

quantity of colchicine contained in 10 gm. of the sample.

293. **According to G. Bredemann,\*** who has studied the various assay methods proposed in the past, the most practical procedure, whether for corm or seeds is to exhaust the powdered drug in the outset by percolation with alcohol of about 70 percent (vol.) and then proceed as follows, adopting with modifications the method of Katz; evaporate a quantity of the tincture corresponding with 5 gm. of drug to 20 mls, add 0.5 gm. of paraffin and 20 mls of water, and heat further until the volume of the solution is reduced to about 15 mls, cool, filter the solution, melt the cake of paraffin with 10 mls of hot water and 1 mil of acetic acid, stir well and when cool pass the solution through the same filter. Transfer the united filtrates to a separator, add sodium chloride enough to saturate and shake out with 20 mls of chloroform, and then with successive 10 mil portions until all alkaloid is removed. Evaporate to dryness, dissolve the residue in a little hot water, filter if necessary, and wash the residue with hot water. Evaporate filtrate and washings to constant weight over sulphuric acid and weigh.

294. **Panchaud† bases an exact assay** of colchicum on the precipitation of colchicine from a solution in chloroform-ether by petroleum benzin. Put into a flask 15 gm. of coarsely powdered colchicum seed with 99 mls of chloroform, shake frequently during 30 minutes, add 6 mls of ten percent water of ammonia, shake thoroughly at intervals during 30 minutes. Measure for the assay 66 mls of the chloroform, representing 10 gm. of drug. Filter, wash the filter carefully with chloroform, evaporate filtrate and washings to complete dryness, dissolve the residue in 0.7 mil dry chloroform, add 1.5 mls dry ether (dehydrated over metallic sodium) then 30 gm. of dry petroleum benzin (boiling point 50° to 60° C.).

\*Apoth. Ztg., Nov. 21, 25 and 28, 1903, 817, 828 and 840,

†Schweiz Wochensch. f. Chem. u. Pharm., 1906, 564.

Filter through an 8 cm. filter, transferring to this the last portions of the precipitate by aid of more petroleum benzin. Redissolve the colchicine in warm chloroform, evaporate once more to complete dryness, dissolve the residue in 15 drops of dry chloroform, add 3 mls of dry ether, and after solution, precipitate the colchicine again by addition of 40 mls dry petroleum benzin. Collect this on a small tared filter, dissolve any floccules adhering to the flask in 5 drops of chloroform, add 1 ml dry ether and 10 mls dry petroleum benzin and collect any precipitate formed on the tared filter. Dry to constant weight and weigh. Add to the weight of the dried precipitate 0.0022 gm. as correction for solubility of colchicine in the quantity of solvent used.

295. **Colchicine** is so feebly alkaline that it cannot be determined by alkalimetric titration with any of the ordinary indicators. The method of Prescott and Gordin (74) may be resorted to, the alkaloid being precipitated with Wagner's reagent after addition of a definite quantity of volumetric acid. Results thus far reported are not very satisfactory.

296. **Titration of colchicine** with Mayer's reagent by Gunnar Heikel's residual method is practicable. Indeed, fairly good results can be reached by simple titration with this reagent in a solution containing 2.5 to 3 percent of sulphuric acid.\*

297. **Assay of Galenical Preparations.** From a fluidextract the alkaloid can be easily extracted by evaporating 10 mls of the fluid nearly to dryness, adding 1 gm. of solid paraffin, heating on the water bath with 15 mls of water added little by little with constant stirring, continuing heat 5 minutes, cooling and transferring the filtered water solution to a separator, treating the cake of paraffin as before with 10 mls of water which is added to that in the separator and shaking out the alkaloid with chloroform. **As an alternative**, dilute 10 mls of the fluidextract with

\*See Lyons' Pharmaceutical Assaying, p. 77, and Lyons' Assay of Drugs, p. 142.

80 mls of water, add solution of lead subacetate in slight excess (i. e. until the fluid has a distinctly sweetish taste), make up to 100 mls with water and filter. Precipitate excess of lead with dry sodium phosphate, filter once more and shake out the alkaloid from an aliquot part and purify as in (292).

## CONIUM

298. **The volatile alkaloid coniine** is the active constituent of poison hemlock, occurring in all parts of the plant, but most abundantly in the immature fruits which were official in U. S. P. VIII. The remedy has fallen almost wholly into disuse. Drug of good quality contains not less than 0.5 percent of ether-soluble alkaloids estimated as coniine, but it deteriorates with age unless special precautions are taken for preserving it. The assay of the drug for total alkaloid presents no special difficulties, provided it be borne in mind that the alkaloid is a volatile substance, and that it is accompanied in the drug not only by certain allied bases, of which no account need ordinarily be taken, but by a notable quantity of ammonia. The quality of a sample of drug may be judged roughly by the "mousy" odor of coniine given off when it is moistened with a solution of potassium or sodium hydroxide.

299. **Assay of Conium fruit.** R. A. Cripps\* exhausts the drug by percolating the powder (5 gm.) with a mixture of dehydrated alcohol 25 mls, chloroform 15 mls and a saturated solution of dry hydrochloric acid gas in chloroform, 10 mls, and then extracting the marc in a soxhlet apparatus 2 hours with the percolate. When cold the solution is shaken out in a separator with two successive portions (25 mls) of distilled water. The aqueous solution is treated with several small portions of chloroform to remove coloring matter and other impurities, and then made alkaline with sodium hydroxide and the alkaloid extracted

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\*Pharm. Journ., (3) 18, pp. 13, 511.

with three successive portions of chloroform. The chloroform is washed with a little alkaline water [why not better pure water? Ed.], neutralized with a solution of hydrochloric acid gas in ether and the solvent evaporated by an air current. The residue of coniine hydrochloride is dried at a temperature not above 90° C. and weighed. Each gm. corresponds with 0.7771 gm. of coniine. The weighing is checked by titration with tenth-normal silver nitrate using potassium chromate as indicator, each mil of the volumetric solution corresponding with 0.01272 gm. of coniine.

300. The assay process may be shortened by treating the washed chloroform solution of coniine with an excess of tenth-normal sulphuric acid, evaporating off the chloroform and titrating the excess of acid with volumetric lime water using methyl red as indicator.

301. **Assay of galenical preparations.** A fluid-extract may be treated as follows: evaporate 15 mls of the fluid with 0.25 gm. of tartaric acid to 5 mls in an Erlenmeyer flask. When cold, add 0.3 gm. of sodium bicarbonate (more if necessary to supersaturate the acid present) and 150 mls of petroleum benzin. Shake well during two minutes (after carbon dioxide is eliminated) let stand half an hour, decant 100 mls, wash this in a separator with 10 mls of distilled water, draw this off into a second separator, shake out with 5 mls of chloroform which, after separation, is to be added to the benzin in the first separator. Add to this 10 mls of tenth-normal sulphuric acid, shake 2 minutes and when separated draw off the acid into a small flask, rinsing at once the tube of the separator with a little distilled water to be added to the flask. Shake the benzin with two successive portions of distilled water (10 mls each) which is also added to the flask. Finally titrate the excess of acid with tenth-normal alkali, subtract the quantity used from 10 and multiply the remainder by 0.01272 to find the quantity of coniine in grammes in 10 mls of the fluidextract.

## DIACETYLMORPHINE

302. **Diacetylmorphine** may be most conveniently standardized by alkalimetric titration. Dissolve about 0.15 gm. of the sample in 5 mls of tenth-normal hydrochloric acid and titrate the excess of acid with lime water (70). Reduce to tenth-normal basis the number of mls of lime water used and subtract this from 5. Multiply the remainder by 0.036919 to find the weight of anhydrous diacetylmorphine present in the sample. Since the product generally contains some  $H_2O$  (about one mol.) it would be better to use the factor 0.038721, representing the monohydrated alkaloid. The result obviously will be correct only if other alkaloids are absent, and if it is present wholly uncombined. Regarding the latter condition there would rarely be any question.

303. To make sure that **foreign alkaloids are absent**, apply the very neat test of the U. S. P. IX. Advantage is taken in this of the fact that diacetylmorphine is split up by hydrochloric acid with formation of morphine hydrochloride. Dissolve the sample (0.5 to 1.0 gm., accurately weighed) in about ten times its weight of water, add half the volume of 10 percent hydrochloric acid and evaporate on the water bath to 1.5—2 mls. Add 20 mls of water, make strongly alkaline with sodium hydroxide and shake out the alkaloid with chloroform (15, 15 and 10 mls). Evaporate the chloroformic solution to dryness, dissolve the residue in 2 mls of tenth-normal hydrochloric acid and titrate excess of acid with lime water (70) using methyl red as indicator. The quantity of this must be not less than the equivalent of 1.5 mls of tenth-normal alkali, the difference between this and 2 mls corresponding with the quantity of foreign alkaloid present. The actual quantity of diacetylmorphine is found by subtracting this amount (calculated as diacetylmorphine) from the amount shown in the titration of (304). Observe that **morphine** as an impurity is not detected by this proced-

ure. If its presence is suspected, dissolve 0.25 gm. of the sample in 25 mls of water by aid of diluted sulphuric acid, add sodium hydroxide in very slight excess and immediately shake out with chloroform continuing the extraction as long as alkaloid is taken out. Determine the alkaloid in the usual manner, but add to the residual solution in the separator sulphuric acid in very slight excess, then neutralize accurately with very dilute ammonia, add one drop of 10 percent solution of ammonia and shake out with a mixture of chloroform and alcohol (2:1, vol.), which will extract morphine if present. See (399). (At least 6 repetitions of the shaking out process will be required to extract the whole of the morphine, which can be determined in the usual method by alkalimetric titration.)

304. **Diacetylmorphine hydrochloride** as supplied by manufacturers generally contains 1 molecule of  $H_2O$ . Assay of the salt is to be carried out on principles just explained. From a sample dissolved in water, the alkaloid is to be extracted by rendering alkaline with ammonia in considerable excess and shaking out with chloroform. Titration factor for the mono-hydrated salt 0.042368. Treat a second sample with hydrochloric acid and extract foreign alkaloids (except morphine) with chloroform, as explained in (303).

305. **Colorimetric determination** of small quantities (e. g. in tablets). In absence of morphine or codeine, approximate estimation may be made by the method of R. Miller.\* Take for the test a quantity of material containing 1 to 3 mg. of diacetylmorphine. Dissolve this in 1 mil of 1 percent sulphuric acid and add 3 mls of a mixture of 24 volumes of strong sulphuric acid, 12 volumes of water and 1 volume of 40 percent formaldehyde solution. Prepare similar mixtures containing resp. 3.0, 2.5, 2.0, 1.5 and 1 mg. of diacetylmorphine. Compare the colors in Nessler tubes, after the mixtures have stood 15 minutes,

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\*Am. Journ. Pharm., 1915, 248.

when it will be seen which mixture most nearly approaches that made from the sample. A second trial will give a close approximation to identity in the colors produced.

## ERGOT

**306. Great confusion has until lately existed** with regard to the active principle or principles of ergot. Each investigator of the subject came to conclusions quite different from those of his predecessors, and so one might take his choice between cornutine, ergotinine, sphacelotoxin, secalintoxin, chrysotoxin, sclerotic (or ergotic) acid, and sphacelic acid, or a combination of two or more of these. Through the researches largely of Barger and his associates, with valuable aid from Kraft, Tanret, a pioneer in these researches, and others, our knowledge of the chemistry and pharmacology of the drug, although confessedly incomplete, has become definite and incontrovertible. The characteristic therapeutic effects of ergot are largely due to the alkaloid ergotoxine, the cornutine of Kobert, the amorphous ergotinine of Tanret and the hydroergotinine of Kraft, differing from the inert ergotinine ( $C_{35}H_{39}O_5N_5$ ) by one added molecule of  $H_2O$ . Ergotinine is itself a crystallizable alkaloid, but forms so far as yet known only uncrystallizable salts, while ergotoxin is uncrystallizable but forms readily crystallizable salts.

**307. Ergot contains** besides alkaloids a number of compounds which occur elsewhere as products of putrefaction, such as cadaverine and putrescine. Among these some have important active properties. They belong to the class of amines derived from amino-acids by the splitting off of  $CO_2$ . Two of these are particularly important, tyramine (derived from tyrosin) resembling epinephrine in that it increases blood pressure, and ergamine which acts powerfully on uterine muscular tissue, coinciding in this particular with ergotoxine, but more active.

**308. It is evident** that a drug containing physiologically active substances differing so widely in char-



acter presents peculiar difficulties as to standardization. Perhaps ultimately this may lead to the disuse of the drug, and the employment instead of its constituent active principles, according to the requirements of the case. For the present, we must be content to base standardization on some one of these active principles and our selection will naturally be of that one peculiar to the drug, viz. ergotoxine—possibly making a requirement of maximum or minimum content of one or more of the other active constituents. It is doubtful whether we can at present formulate an assay process which will isolate, and extract the whole of the ergotoxine, but such a process may no doubt ultimately be devised.

309. Tyramine and ergamine are readily **soluble in water**, but are not easily extracted in a state of purity. The former can be obtained in crude form by shaking out the aqueous solution with amyl alcohol, having first removed other substances by shaking out with other immiscible solvents. (Ether also in liberal quantity will extract it.) The fact that this compound gives with Millon's reagent an intense color, suggests the possibility of a colorimetric test, if it can be shown that the amyl alcohol extract does not contain other substances which give a similar color with Millon's reagent.

310. **Ergamine yields with picrolonic acid** a very sparingly soluble precipitate, suggesting a possibly practical method of separating this principle from accompanying impurities. Satisfactory assay processes for both tyramine and ergamine can no doubt be worked out if it shall be thought necessary.

311. **Biological assay of ergot.** There are three methods practised for ascertaining experimentally the activity of preparations of ergot. That which is simplest is observation of the effect produced on a cock's comb by a certain dose of the drug, given preferably by intramuscular injection. If Leghorn fowls of pure breed are used for the experiment, results of the test are said to be quite satisfactory. This certainly is not true in the case of the ordinary barnyard fowl. Preference is given this test, first because

it shows only ergotoxin, tyramine and ergamine producing no similar effect. Second, the test requires no elaborate apparatus. Third, for this test we have a standard of comparison in crystallized ergotoxin phosphate. With our present knowledge of the chemistry of ergot, it should be possible to prepare a fluidextract that will represent the physiological activity of the drug. Such a preparation would serve as a standard of comparison by any of the methods of biological assay. Unfortunately fluidextracts of ergot deteriorate more or less rapidly if kept under ordinary conditions. It is possible, however, according to Pettinger and Vanderkleed\* to keep such preparations for long periods with very little deterioration if preserved in vacuo.

**312. The second method** of testing ergot biologically is by its influence on blood pressure in animals like the dog. For such a test the equipment of a "biochemical" laboratory is essential, together with expert skill in this kind of work. The test has a quantitative definiteness that is lacking in the cock's comb method, but it remains yet to be shown that the pressor effect produced is quantitatively proportioned to the therapeutic activity of the drug. Inasmuch as we know that tyramine acts much more powerfully than other known constituents of ergot on the blood pressure, it seems certain that the assay cannot give anything like an accurate measure of the value of a sample, particularly as the drug is known to contain compounds which diminish blood pressure. There are, however, some whose wide experience entitles their opinions to weight, who maintain that the method does in practice indicate better than any other the medicinal potency of the sample assayed, and who fix definitely a specific effect for a standard drug. When administered to dogs in the proportion of 0.08 gm. per kilo of body weight†, it should produce, they say, an increase of 30 mm. in blood pressure.

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\*Journ. A. Ph., A., 1912, p. 799.

†P. S. Pittenger, Biochemic Drug Assay methods, p. 65.

313. **The third method** of biological assay is based on the fact that ergotoxin and ergamine affect uterine muscular tissue in certain definite ways. The details of the assay processes which have been adopted by different authorities need not be entered into here, since a definite standard based on clinical observations remains yet to be fixed. Furthermore, the technique of such an assay calls for even greater operative skill than the blood pressure assay.

314. It remains to hit upon some simple method of judging the activity of a sample of the drug by effects produced on a human subject by an ordinary medicinal dose, e. g. by contraction of minute blood vessels in the eye, or by sensations caused by diminished blood supply to the brain.

### GELSEMIUM

315. **It is unfortunate** that the chemistry of this drug, which possesses unquestionably important therapeutic properties, is as yet only imperfectly understood. It may be assumed that its medicinal activity resides in its alkaloids. One of these, known in America as gelsemine, is easily obtained in crystalline form, and forms also crystallizable salts. The crystals melt at 175-178° C. and have the formula, according to C. W. Moore\*  $C_{20}H_{22}O_2N_2$ . It is physiologically active, exerting its influence according to F. P. Chillingsworth† on the central nervous system, and only secondarily on the heart. The drug contains in larger proportion an uncrystallizable alkaloid, commonly known in America as gelseminine‡. There is reason to believe that this consists of at least two distinct alkaloids, but attempts to effect a complete separation of them, or to obtain either in a form sufficiently pure to ascertain its molecular formula have not thus far been successful. That which is settled is that the amorphous alkaloid is

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\*Journ. Chem. Soc., 1910, 2229.

†Journ. Am. Pharm. Assoc., 1914, 315-21.

‡In Germany and perhaps elsewhere in Europe the crystallizable alkaloid of gelsemium is called gelseminine, the amorphous gelsemine.

several times as active as gelsemine, but produces quite different effects.

315½. Stevenson and Sayre\* report the discovery in small proportion of a second crystallizable alkaloid in gelsemium, for which they suggest the name *sempervirine*. Its physiological activity has not been studied. It is evident that under these circumstances it is impossible to fix any standard that will assure to the physician uniform therapeutic activity in the preparations of this drug.

316. It is not a difficult matter to determine by assay conducted after type process I (102) the total alkaloid contained in the drug. The immiscible solvent to be chosen is a mixture of chloroform and ether (equal volumes). The drug contains a fluorescent principle, aesculetin monomethyl ether, called for short by Eykman, scopoletin, the "gelseminic acid" of Wormley. This is removed from acid solutions by shaking out repeatedly with an immiscible solvent, which, however, takes out an appreciable quantity of the alkaloid, to be recovered by shaking the solution again with diluted acid.

317. **For assaying the fluid extract**, John R. Rippetoe† directs the following procedure, based on Webster's method: Transfer 15 mls of the sample to a 200 mil graduated cylinder, add 10 mls of water, 5 mls of sodium hydroxide T. S. and 150 mls of chloroform-ether mixture (4:1), shake frequently during one hour, set aside and when the chloroform-ether layer has become clear, note its volume and draw off two-thirds, representing 10 mls of the sample. Transfer to a separator and extract the alkaloids with 1 percent sulphuric acid; make the solution alkaline with sodium hydroxide and shake out with chloroform-ether (4:1). Wash each portion of the chloroform-ether with water and filter through a dry filter. Unite the solutions, distil (or evaporate) to one-third and extract the alkaloids by shaking out first with 5 mls of tenth-normal sulphuric acid diluted

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\*Journ. Am. Pharm. Assoc., 1915, 60-2.

†Proc. Am. Pharm. Assoc., 1910, 1061.

with 10 mls of water, then with 3 successive portions of distilled water (10, 10, 5 mls). Finally titrate the combined aqueous solutions with fiftieth normal potassium hydroxide using 20 drops of cochineal indicator. (Preferably use tenth-normal hydrochloric acid and lime water with methyl red or cochineal as indicator. Provisional factor, 0.0322.—Ed.)

## HYDRASTIS

**318. Active constituents.** The drug contains two principal alkaloids. The more abundant is that to which it owes its bitterness and its deep yellow color, berberine, a constituent of many plants besides golden seal. The other, which is characteristic of this drug and distinctively its active principle, is hydrastine. Berberine is soluble in chloroform, but practically insoluble in ether and forms readily crystallizable salts, many of which are only sparingly soluble in water, particularly in presence of free acid. Its iodide is quite insoluble in water. Hydrastine is very freely soluble in ether, sparingly soluble in petroleum benzin, is easily crystallizable, but is of very feeble alkalinity and forms salts which cannot easily be crystallized. Hydrastinine, a derivative of hydrastine, may be present in the drug in appreciable quantity, recognized by the fluorescence of its solutions.

**319. Assay of the drug.** Put into a flask 6 gm. of hydrastis in fine powder. Add 180 mls of ether, shake, let stand 10 minutes, then add 5 mls of water of ammonia, shake continuously half an hour, or at frequent intervals during two hours, add 10 mls of water, shake, and when clear, pour off 150 mls, equivalent to 5 gm. of drug. Recover the ether by distillation, dissolve the residue by gently warming with 10 mls of 0.5 percent hydrochloric acid, filter into a separator and wash the filter twice with hydrochloric acid of the same strength. Add 40 mls of ether and water of ammonia to strong alkaline reaction, shake well during two minutes, add 35 mls of petroleum benzin and shake once more. Add

1.5 gm. powdered tragacanth, shake until the ethereal solution is perfectly clear, decant 60 mls, equivalent to 4 gm. of drug. Evaporate to dryness in a tared beaker and weigh or titrate.

#### Alkalimetric

##### 320. Alkalimetric Titration of Hydrastine.

The alkaloid is so feebly alkaline that most indicators give unsatisfactory results. Methyl red or litmus may be used, but the end point is not sharp. Methyl orange is recommended by some. Better results may be obtained by Gordin's method (74). The alkaloid is so insoluble in water that a titration may be made in a solution free from alcohol without use of an indicator. The solution, containing 50 to 100 mg. of hydrastine, should not measure more than 20 mls. Add the volumetric alkali (lime water) until a faint persistent turbidity is produced. Take this for the end point of the titration, but correct the result by adding for each mil of solution (at the end of the titration) 0.0007 gm. Each mil of tenth-normal acid corresponds with 0.0383 gm. of hydrastine.

321. Possibly more satisfactory will prove determination of the alkaloid by conversion into hydrochloride and volumetric determination of the chlorine (see 76 to 80). Each mil of tenth-normal solution of silver nitrate corresponds to 0.0383 gm. of hydrastine.

322. **Another alternative** is the periodide assay of Prescott and Gordin (84), which is said to give fairly good results. Christensens' iodometric titration (75) does not succeed with hydrastine.

323. **Determination of Hydrastine as Picrolonate.\*** Having extracted the hydrastine with a mixture of ether 5 parts (by weight) and petroleum benzin 1 part, evaporate the solution (representing about 0.1 gm. of hydrastine or 5 gm. of hydrastis) to one-half, add 10 mls of tenth-normal alcoholic solution of picrolonic acid (0.0264 gm. in each mil), stir well and let stand 24 hours. Collect the precipitate on a Gooch filter, wash with 1 mil of alcohol-ether

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\*Matthes and Rammstedt, Arch. Pharm., 245, 112-32; Inst. f. Pharm. u. Nahr-Chem., Univ. Jena.

mixture, dry 30 minutes at 105° C. and weigh. Each gm. corresponds with 0.572 gm. of hydrastine.

**324. Determination by saponification with Hydriodic acid.** According to J. Gesell\* hydrastine can be determined by saponification with hydriodic acid (sp. gr. 1.71) in a Benedikt apparatus. One gramme of hydrastine is the equivalent of 1.248 gm. of silver iodide. The author determines berberine in the same manner, 1 gm. being the equivalent of 0.987 gm. of silver iodide.

**325. Determination of Berberine.** The drug (10 gm.) may be exhausted with strong alcohol in an extraction apparatus,† the solution made up with alcohol to 100 mls. To 25 mls of this solution add 1.3 mls of hydrochloric acid (U. S. P.), let stand in a refrigerator 24 hours, then transfer the crystals of berberine hydrochloride to a pair of counterpoised filters, wash with a mixture of equal volumes of ether and alcohol until all free acid is removed, press, dry at 105° C. and weigh. Multiply weight by 0.9018 to find weight of berberine.

**326. Alternative methods.** L. David‡ directs to precipitate (presumably from an aqueous solution) berberine and hydrastine with potassium bismuth iodide solution, dissolve the hydrastine precipitate with acetic ether, liberate the berberine in the residue with 10 percent solution of sodium hydroxide, shake out with ether-chloroform, evaporate, dry and weigh. In a fluidextract from which the hydrastine has been removed by shaking out with ether and ammonia, an approximate determination of berberine may be made by diluting with water, filtering if necessary, acidulating with hydrochloric acid, precipitating with Mayer's reagent in slight excess, collecting on a pair of counterpoised filters, drying and weighing. One half the weight may be taken as approximately the weight of the berberine.§

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\*Chem. Ztg., 1914, No. 50, 541.

†F. A. Thompson, Proc. Mich. State Pharm. Assoc., 1893; Am. Journ. Pharm. 1893, 371.

‡Pharm. Post, 1915, 48, 1, 21.

§Lyons' Assay of drugs, p. 179.

326½. A neat method of determining berberine is that of H. M. Gordin,\* in which the alkaloid is made to combine with acetone, and is weighed in that condition. The berberine is first precipitated from aqueous solution by adding an excess of a 10 percent solution of potassium iodide. The precipitate is washed with a 2 percent solution of potassium iodide, rinsed with "a little" water into an Erlenmeyer flask, the mixture brought to a temperature of 65° C., and acetone, equal in volume to one-third of the fluid in the flask, is added and the mixture shaken 10 minutes. Five mls of a 10 percent solution of sodium hydroxide are then added and the mixture is shaken 10 minutes or so until the yellow color of the precipitate is discharged (heat being again applied if necessary). The mixture is cooled and water added to bring the volume to nine times that of the acetone present. The flask is set by overnight, the acetone-berberine is collected on a tared filter and dried, at first under diminished pressure, finally at 105°C. Each gm. of the berberine compound corresponds with 0.853 gm. of berberine. Add for each 100 mls of solution 0.00273 gm. as correction for solubility.

327. **Assay of the fluidextract.** A satisfactory procedure is that of A. W. van der Haart†. Mix 10 mls of the fluid with 20 mls of water, and boil until reduced to 10 or 12 gm. Add 4 mls of diluted hydrochloric acid. When cold add water to bring the weight to 20 gm., add 1 gm. of purified talc and filter. Transfer 10 gm. of the filtrate to a 100 ml flask, add 7 mls of water of ammonia and 25 mls of ether, and shake for 2 minutes. Add 25 mls of petroleum benzin (b. p. 60-80°C.) shake half a minute, add 2 gm. of powdered tragacanth and again shake thoroughly. Forty mls (equivalent to 4 mls of the fluidextract) are transferred to a flask, 5 mls of petroleum benzin are added and the solution is submitted to distillation until 35 mls have been recovered. The flask is corked and set by 18-24 hours in a cool

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\*Proc. Am. Pharm. Assoc., 1891.

†Arch. Pharm., 1901, 239, 638-45.



place. The fluid is then carefully decanted, the crystals washed with 2 mls of light petroleum benzin, dried on the water bath and weighed. The method is substantially that of the German pharmacopoeia, except that there the alkaloidal solution is simply evaporated to dryness and the residue weighed.

328. **The U. S. P. IX assay process** is open to criticism, particularly in that the alcohol is not driven off before extracting with ether. It is not stated that the product of the assay is hydrastine; it is spoken of simply as the ether-soluble alkaloids of hydrastis. Nothing is said of the possible presence in the alkaloid of hydrastinine, a product derived by oxidation from hydrastine, characterized by a strong blue fluorescence. Inasmuch as the therapeutic properties of the two alkaloids are quite different, this question should be investigated and the assay process should not be considered satisfactory if it did not practically exclude the derivative alkaloid.

## HYOSCYAMUS

329. **The drug contains a very small proportion** of alkaloid (a mixture of mydriatic alkaloids.) The assay processes of the various pharmacopoeias are the same as those used for belladonna leaves, except that a larger quantity of the drug (as much as 20 or 25 gm.) is taken for an assay. It has been shown by O. Anselmino\* that the assay process of the German pharmacopoeia fails to extract the whole of the alkaloid, although an identical process succeeds with belladonna leaves. An extract made with diluted alcohol, by the official (German) assay showed the drug to contain 0.112 percent of alkaloid, while direct assay gave only 0.071 percent. A similar discrepancy has not been observed where the assays have been made by the methods of the U. S. P., but further study should be made of the subject. In any case, it will be well to increase the quantity of primary solvent, as well as of drug, in the assay. Preferably follow the procedure of (102) using 20

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\*Arch. de Pharm., 1913, 361.

gm. of drug, with 200 mls of primary solvent (chloroform 1 volume, ether 4 or 5 volumes), percolating the marc to obtain 250 mls of fluid. See (196).

330. **The observation has been made** by Dr. J. M. Francis that in the case of an old fluidextract, it is necessary to use a mixture of chloroform with alcohol in place of chloroform alone in the final extraction of the alkaloid. If this is the fact, we are driven to conclude that the alkaloid undergoes some change with age, and that hence an assay cannot be depended upon to give a correct indication of the medicinal activity of such a preparation.

331. In assaying **galenical preparations of henbane**, preference should be given to the lead subacetate process, making up the solution so that 100 mls of it shall represent 15 mls of the fluidextract, 150 mls of the tincture or 4 gm. of the extract. See (205) and (207).

332. Otherwise the alkaloids may be precipitated from the acid aqueous solution with silicotungstic acid, as recommended by Javillier and Guerilhault\*. The precipitate is to be collected on an ashless filter, washed with 1 percent hydrochloric acid, dried and ignited. Multiply its weight by 0.4064 to find weight of alkaloid calculated as atropine. Correction for solubility, 0.0048 gm. for each 100 mls of mother liquor and washings.

333. **The international protocol** fixes no standard for hyoscyamus or for any of its preparations. The pharmacopoeias give for the drug either no standard or a standard ranging from a minimum of 0.055 (U. S. P.) to 0.1 percent (Swiss); for the extract, the standard, where any is given, varies from 0.22 (U. S. P.) to 0.5 percent (German).

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\*Bull. Sci., Pharm., 1911, 93.

## IPECAC

334. **Both Brazilian and Cartagena ipecac** contain the three related alkaloids, emetine, cephaeline and psychotrine, the proportions varying in the different varieties. The molecular formulas of the alkaloids according to F. H. Carr and F. L. Pyman\* are resp. for emetine  $C_{29}H_{40}O_4N_2 = 480.34$ ; for cephaeline  $C_{28}H_{38}O_4N_2 = 466.32$ , and for psychotrine  $C_{28}H_{36}O_4N_2 = 464.31$ . These formulas differ from those commonly accepted, and certainly are not to be regarded as final. The U. S. P. IX makes the formulas (mono-acid) for the two former, resp.  $C_{15}H_{22}O_2N = 248.19$  and  $C_{14}H_{19}O_2N = 233.16$ . Psychotrine is present only in small proportion and is not known to have important therapeutic properties. The medicinal effects produced by ipecac are generally credited to emetine and cephaeline, the latter more active as an emetic than the former, which, however, is believed to be medicinally the more important. It is customary in assays of the drug to determine simply "ether-soluble alkaloids," i. e. emetine and cephaeline combined.

335. **A simple and trustworthy assay process**, according to Dr. G. Fromme†, is that proposed by Panchaud, essentially as follows; Put into a flask 5 gm. of finely powdered ipecac with 150 mls of ether and 5 mls of water of ammonia, and shake together frequently and vigorously during one hour. Let stand 5 minutes to settle, decant 120 mls of the clear ether solution, representing 4 gm. of drug. Distil to 20 mls, add 5 mls of alcohol, 3 drops of haematoxylin solution and 10 mls of water, and titrate with twenty-fifth-normal acid. When near the neutrality point, add 30 mls more of water, and complete the titration. Each mil of twentyfifth-normal acid corresponds with 0.006 gm. of the mixed alkaloids, but see (336) and (338).

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\*Proc. Chem. Soc., 1914, 157.

†Pharm. Ztg., Sept. 17, 1904, 791.

**336. Dr. Fromme finds this method** of titration to give results a little too high, and advises to distil off the whole of the ether before titration, presumably dissolving the alkaloid in an excess of volumetric acid and titrating back with volumetric alkali. Care must be taken if this plan is adopted not to overheat the alkaloidal residue. It is best to drive off the last of the ether by an air blast. If the residue is overheated it yields a colored solution, and there is a notable loss (it may be a very large loss) of alkaloid. The most satisfactory indicator for this titration is methyl red. Haematoxylin also gives good results. This indicator, however, ought never to be kept in solution. Instead use a minute quantity, less than a milligram, of the dry powder. Ether or chloroform may be present, but in such case, the mixture must be shaken well after each addition of the volumetric solution. Uranin and iodeosin are good indicators, but can be used only in conjunction with ether, and the essential shaking of the mixture after every addition of reagent, makes the titration a tedious operation. Cochineal may be used if the solution is not too deeply colored, but the inexperienced or partially color-blind have difficulty in noting the color change which marks the end of the titration. The titration factor commonly accepted for the mixed alkaloids is 0.024. If, however, Carr and Pyman are right with regard to the molecular weights of emetine and cephaeline, the correct factor is 0.02367, i. e., the mean between 0.02402 and 0.02332

**337. Quite similar** to the method of Dr. Fromme is that of G. Frerichs and N. de Fuentes Tapis\*. Shake together at intervals during two hours, 6 gm. of finely powdered ipecac, 90 mls of ether and 5 mls of water of ammonia (or of a solution of sodium carbonate, (1:3). Add 10 mls of water, shake and decant 75 mls of the ether solution, representing 5 gm. of drug. Evaporate to one half, shake out the remainder with 10 mls of tenth-normal hydrochloric acid followed by two portions (10 mls each) of water. Dilute the mixed acid solutions to 100 mls, add 25 mls of

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\*Arch. d. Pharm., Sept. 10, 1902, 401.

ether and titrate with tenth-normal potassium hydroxide, using iodeosin as indicator. Titration factor taken as 0.0241.

**338. Pharmacopoeial assay processes** for ipecac are commonly conducted on the general plan of the Keller method, the primary solvent in some cases simply ether, which is rational—in others ether-chloroform, which will extract at least a part of the psychotrine—a doubtful advantage. The alkali chosen may be ammonia, sodium carbonate, even sodium hydroxide, the last in case the object of the assay is to determine emetine alone. In most cases the alkaloid is determined by titration, the details varying greatly, the titration factor ranging from 0.0240 to 0.0254. In a few cases the alkaloid is weighed. Naturally, results of an assay will vary considerably. The standard also varies, although two percent of total alkaloids or of ether-soluble alkaloids is the figure adopted by the majority.

**339. Assay process of U. S. P. IX.** The usual routine is followed except that ether alone is used as the immiscible solvent. The titration factor is 0.024, the indicator being the usual cochineal. The standard for the drug, which includes the Cartagena as well as the Rio variety, is a minimum of 1.75 percent of ether-soluble alkaloids. For the fluidextract of ipecac, the standard is 2 percent (1.8 to 2.2 percent permissible). The discrepancy is due to the fact that the drug imported into America contains only exceptionally as much as 2 percent of ether-soluble alkaloids whereas the standard of the international protocol for fluid-extract of Ipecac is practically 2 percent.

**340. Determination of both emetine and cephaeline.** Inasmuch as the medicinal properties of emetine and cephaeline are quite different, evaluation of a sample of ipecac requires a knowledge of the proportions present of the two principal alkaloids. A. G. C. Patterson\* proposes the following assay process providing for the two determinations. Put

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\*Pharm. Journ., 1903, 73-75 & 101-2.

into a stoppered bottle 12 gm. of the powdered drug with 120 mls of a menstruum composed of chloroform 1 part (volume?), amyl alcohol 1 part, and ether 3 parts. Add 10 mls of water of ammonia (or 10 mls of a sodium carbonate solution (1:3), shake together one hour, then add 10 to 15 mls of water to aggregate the powder. After settling, decant 100 mls of the ethereal solution (representing 8.33 gm. of drug), evaporate to one-half and shake out with 15 mls (or an excess) of tenth-normal hydrochloric acid, followed by 3 portions of water (3 mls each). Add to the combined aqueous solutions 2 mls of normal potassium hydroxide solution, and shake out with ether (15, 10, 10, and 5 mls), which extracts the emetine, leaving nearly all the cephaeline in the aqueous solution. Shake out the mixed ethereal solutions with twentieth-normal potassium hydroxide (10, 5 and 5 mls) to remove residual cephaeline, shake out these alkaline solutions with 10 mls of ether to recover traces of emetine, combine the ethereal solutions, evaporate and weigh the residue as emetine, or titrate it (factor 0.0248 [0.0240? Ed.]). Unite all the aqueous alkaline solutions, acidify with hydrochloric acid, then render alkaline with ammonia and shake out with ether-chloroform 1:6 (20, 10, 10 and 5 mls), evaporate, dry the residue and weigh as cephaeline (or titrate, using methyl orange as indicator, factor 0.0234 [0.0233? Ed.]).

**341. Assay of the fluidextract.** Troublesome emulsions are apt to form if the usual routine is followed in this assay. One plan which often succeeds is to use only 2 mls of the fluidextract, adding to this 5 mls of diluted alcohol, or 3 mls of alcohol and 2 to 5 mls of water (as much, within those limits, as practicable without precipitation). Then add 40 mls of ether, shake together, add, one drop at a time, shaking after each addition, diluted water of ammonia (about 2 percent strength). When ammonia is in excess, note whether clots of precipitated alkaloid have failed to dissolve in the ether. These will generally adhere to the sides of the separator. Draw off the aqueous fluid (through a pledget of purified

cotton if it contain solid particles) and decant the ether into a beaker. Treat the cotton with 3 mils of 3 percent sulphuric acid with which also the clots of alkaloid in the separator are dissolved; 20 mils of ether, with which the first alkaline solution has been cautiously shaken out, is added to the separator, followed by dilute ammonia in excess, and the mixture is carefully shaken so that the reprecipitated alkaloid goes into solution in the ether, which is separated and added to the first ether solution. The alkaline solution in the separator is shaken out once more with ether (15 mils), the united ether solutions are evaporated, by aid at the end of an air current, and the residual alkaloids are titrated as in assay of the drug.

**342. The gravimetric determination** is preferably made by dissolving the crude alkaloid (0.15 to 0.3 gm.) in a slight excess of 5 percent sulphuric acid, diluting with water to 50 mils and adding 5 percent solution of ammonia to incipient precipitation. Continue the cautious addition of weak ammonia (1%) with constant stirring as long as litmus indicator shows distinct acidity. The precipitate thrown down contains very little alkaloid. It is to be separated by filtration, washed with several small portions of cold water and rejected; the washings are to be added to the filtrate, which is to be made alkaline with ammonia and shaken out with ether-chloroform to obtain an alkaloid sufficiently pure for weighing.

**343. A simpler procedure** is to add to 4 mils of the fluidextract 4 mils of alcohol and 4 to 6 mils of water, then 100 mils of ether and, drop by drop, with constant shaking, five percent ammonia until in distinct excess. Shake together vigorously during two minutes, let separate, decant the ethereal solution into a measuring cylinder and determine the alkaloid in an aliquot portion of it as in the assay of the drug. (If any clots of alkaloid have escaped solution, dissolve these in a little alcohol and add to the original ether solution before taking the aliquot). Observe that where alcohol has been added in this assay, the alkaloid carries considerable impurity, including possibly

psychotrine. It is well to purify it by dissolving it in 5 mls of diluted sulphuric acid, shaking out once with 15 mls of ether to remove impurities, then rendering alkaline with ammonia and shaking out with ether (20, 15, 15 and 10 mls).

343½. The U. S. P. IX adopts the sawdust expedient of (112) and the plan is a good one. Cheese cloth may be substituted for the purified sawdust (113).

344. **Alternative Process**, proposed by W. B. Cowie\*, applicable also to a large number of liquid extracts. Put into a flat bottomed dish 10 mls of the fluidextract, add 5 mls of normal solution of oxalic acid and 10 mls of water, evaporate to 5 mls, add 20 mls of water with 5 mls of the oxalic acid solution, stir well and filter through a pledget of purified cotton into a measuring cylinder. Wash the dish with 10 mls of water and 1 ml of the oxalic acid solution, passing this through the pledget of cotton into the measuring cylinder. Add 1 ml of liquor ferri dialysatus B. P. 1885, make up with water to 50 mls, shake well and set aside to separate. Put into a separator 25 mls of the filtered solution, representing 5 mls of the fluidextract, add excess of ammonia and extract with a mixture of ether and chloroform in equal volumes (20 and 20 mls), then with two portions of chloroform (10 mls). Distil off the solvents, dry to constant weight at 80° C., then dissolve in tenth-normal hydrochloric acid and titrate excess of acid with twentieth-normal sodium hydroxide, using cochineal as indicator. Factor 0.0244.

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\*Pharm. Journ., 1918, 433-7.



**LOBELIA**

345. **Lobeline, the alkaloid of Lobelia**, is described as a liquid or at least a semi-fluid substance. It seems to be commonly assumed that it is volatile at ordinary temperatures in a notable degree, and until the question has been authoritatively settled, it is as well in dealing with it to avoid possible loss by volatilization. The assay may be conducted by (102) using ether as the immiscible solvent. The final ethereal solution is to be allowed to evaporate spontaneously, dried in a desiccator over sulphuric acid and weighed. It may be determined by titration (70), each mil of tenth-normal acid corresponding with 0.028519 gm. of lobeline.

346. **Vanderkleed\*** adopts the same general method, using sodium carbonate in place of ammonia to set free the alkaloid, and shaking out with chloroform instead of ether (although he uses "ether and alcohol" with ammonia as the primary solvent). The chloroformic solution is placed in a tared crystallizing dish containing 30 mls of ether saturated with hydrochloric acid gas, the solvent is evaporated off on a steam bath and dried in an air-bath to constant weight. After weighing, the chlorine in the residue is determined by the Volhard method of titration. Each mil of tenth-normal silver nitrate corresponds with 0.03217 gm. of anhydrous lobeline hydrochloride or 0.028519 gm. of anhydrous lobeline. The drug may be expected to yield 0.5 to 0.7 percent of alkaloid; the seed 0.35 to 0.45 percent. The U. S. P. provides no alkaloidal standard for the drug.

347. **Assay of fluidextracts**, tinctures, etc. For hydroalcoholic preparations adopt the procedure of E. L. Patch†. Put into a separator 5 mls of a fluid-extract with 5 mls of water, add 10 mls of ether and 1 mil of five percent sulphuric acid, shake and separate, rejecting the ether; shake out the acid solution

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\*Journ. Am. Pharm. Assoc., 1916, 713.

†Lyons Assay of Drugs, p. 190.

with two additional portions of ether. Make alkaline with ammonia and shake out the alkaloid with ether, finishing the assay as in (345). In case of a tincture, evaporate 50 mls to about 15 mls, add 1 mil of five percent sulphuric acid and 15 mls of ether, shake together and separate and complete the assay as above (line 6).

348. An **acetic fluidextract** is to be shaken out with 3 successive portions of ether (10 mls) which are to be washed with water to recover any alkaloid that may have been taken out. The washings are to be added to the residual acid solution in the separator, ammonia is to be added in excess, and the alkaloid extracted by shaking out with ether.

### NUX VOMICA 2.59.

349. The **medicinal activity of nux vomica** and the allied drug ignatia is due chiefly to the strychnine they contain. It is true that brucine has a share in that activity but it is comparatively unimportant, and the relative proportion in which these alkaloids are present is so nearly uniform in the respective drugs that the percentage of strychnine furnishes a practically correct measure of the therapeutic value of a sample of either drug. Obviously this cannot be taken as a basis of exact comparison between a sample of nux vomica and one of ignatia, but for such comparison, the content of strychnine is a better criterion than that of total alkaloid, since in nux vomica strychnine constitutes about 40 percent, in ignatia more nearly 60 percent, of the total alkaloids.

350. Unfortunately the **international standards for nux vomica** and its preparations are based on total alkaloids, and these have therefore become almost universally official. The British Pharmacopœia adheres to a strychnine standard. The U. S. P. IX abandoned the strychnine standard to conform to international requirements. It is to be hoped that in time the more rational standard will receive international recognition.

**351. Assay of the crude drug.** Follow type process No. I (102), using 15 gm. of drug in No. 40 powder; primary solvent 150 mls of a mixture of ether 4 volumes, chloroform 1 volume. After addition of ammonia, shake the mixture well at frequent intervals during one hour, or preferably shake continuously half an hour, then let stand one hour. Use 100 mls of the ethereal solution, equivalent to 10 gm. of drug. Dissolve alkaloidal residue in 10 mls of tenth-normal hydrochloric acid and use as indicator preferably methyl red. Each mil of tenth-normal acid is taken to correspond with 0.0364 gm. of the combined alkaloids of nux vomica. Since the proportion of strychnine in the alkaloids is nearer 40 than 50 percent the correct factor is more nearly 0.037.

**352. N. B.** Remember that in shaking out the alkaloids of nux vomica from the primary solvent, hydrochloric acid must not on any account be substituted for sulphuric acid (50).

**353. For determination separately** of strychnine and brucine proceed as follows: Put into a flask 20 gm. of the drug in No. 40 powder, add 200 mls of a mixture (cooled to room temperature) of 160 mls of ether with 55 mls of chloroform. After 10 minutes add 10 mls of water of ammonia. Shake the flask frequently (every 3 minutes) during one hour, let stand one hour, then add 20 mls of water, shake, add about 2 gm. of powdered tragacanth and shake once more. When clear, decant one portion of 50 mls for determination of total alkaloids and another of 100 mls for determination of strychnine. Shake out the first portion with 3 percent sulphuric acid, heat the acid solution in a small beaker with 1 gm. of hard paraffin, stir well, allow the paraffin to congeal, transfer the acid solution to a separator, melt the paraffin with several small successive portions of water until practically freed from bitterness. Render the combined aqueous solutions alkaline with water of ammonia and shake out with chloroform. Evaporate off the solvent, dry at 100° C. to constant weight and weigh as total alkaloids from 5 gm. of

drug. Dissolve the alkaloids in 5 mls of tenth-normal hydrochloric acid and determine residual acid by titration with lime water (70). Each mil of tenth-normal acid corresponds to 36.4 mg. of "total alkaloids" of *nux vomica*, according to U. S. P. IX; 36.9 is more nearly the correct factor. See (351).

354. **Extract the alkaloids** from the second portion of the primary solvent by shaking out with sulphuric acid, wash the acid solution twice with chloroform (15 mls), add excess of ammonia and shake out with several successive portions of chloroform (20, 15, 10 mls or q. s.). Evaporate the alkaloidal solution to dryness, dissolve the residue in 15 mls of 3 percent sulphuric acid by aid of a gentle heat. Cool the solution to 20° C.\* and add to it 1.5 mls of strong nitric acid and 1 mil of a 5 percent solution of sodium nitrite in water,† stir the mixture well and let it stand exactly 10 minutes, to destroy brucine. Transfer to a separator, rinse the container with 15 mls of chloroform and several successive portions (5 mls) of a 15 percent solution of sodium hydroxide. Add then sufficient of the same solution to render the mixture strongly alkaline (indicated by the appearance of permanent turbidity) and extract the alkaloid by shaking out with successive portions (about 15 mls each) of chloroform. Evaporate the combined alkaloidal solutions to about 5 mls, add 5 mls of alcohol, and continue evaporation, by aid of an air current, just to dryness (guarding against decrepitation of the strychnine crystals by too rapid heating). Finally dissolve the strychnine in 5 mls of tenth-normal acid and titrate the excess of acid with lime water (factor 0.0334). We now have the data for determining the quantity of strychnine contained in 10 gm. of drug and the quantity of combined alkaloids in 5 gm. of drug.

\*The British Pharmacopoeia prescribes a temperature of 50° C., but much strychnine may be lost at that temperature by oxidation.

†In absence of sodium nitrite, saturate the nitric acid with nitrous compounds by putting into a test tube one drop of alcohol, adding the acid and allowing the reaction to go on until red fumes begin to appear; add then 1.5 mls of water, and pour the acid at once into the strychnine solution.

Multiply the latter by two and from the product subtract the former to find the brucine in 10 gm. of drug. Check the result by result of titration of the combined alkaloids and further by re-extraction and weighing of the strychnine.

**355. Abbreviated assay** for total alkaloids and strychnine. Extract the drug repeatedly with petroleum ether (preferably in a soxhlet apparatus) to remove fatty matter. Assay the residue by the method of (102), but weigh the mixed alkaloids (from 10 gm. of drug) instead of titrating them. Dissolve the alkaloids as directed in (354) and determine strychnine by titration. Brucine will be found by difference.

**356. Official assay processes** for nux vomica in the leading pharmacopoeias, show remarkable unanimity in choice of method as well as in the standard adopted (2.5 percent combined alkaloids). Twelve of these assay processes have been brought into comparison by Dohme and Engelhardt\*, who reported results of assays made by the several methods on the same sample of nux vomica. The yield by five of the official methods was close to 2.50 percent, by two methods it was 2.80 percent or above (but one of these was identical with one of those which yielded 2.50 percent). The remaining five methods yielded resp. 2.38, 2.20, 2.08, 2.06 and 1.09 percent.

**357. Differences in detail** of the official methods relate to (1) the proportion of primary solvent to drug, in general 10: 1 (vol.) but ranging as high as 25:1 (wt.); the larger proportion does not give an increased yield; (2) relative proportion of ether and chloroform, generally about 4:1 (vol.); the U. S. P. ~~2:1~~ is exceptional but this does not seem to affect results; (3) the choice of alkali between ammonia and sodium hydroxide; in no case, where the latter is used, were results low, but low results cannot be ascribed to the use of ammonia, which on general principles is preferable; (4) the time given for extraction of the alkaloid by the primary solvent. This ranges in

\*Proc. Am. Pharm. Assoc., 1910, 829-50.

processes giving good results from 15 minutes of continuous shaking with one hour of subsequent maceration, to 3 hours of occasional shaking with 1 to 10 hours of maceration. There seems to be no advantage in prolonged maceration. Low results may be expected where the period for frequent shaking is less than an hour (2 hours preferable) or for continuous shaking less than half an hour; (5) the preliminary treatment of the drug with petroleum benzin to remove fatty matters; this might possibly occasion some loss of alkaloid; on the other hand the presence of fatty compounds may affect titration results; no conclusion can be drawn from the results reported, which, however, show closer agreement between gravimetric and volumetric results than was to be expected; (6) choice of the acid to be used in shaking out the alkaloids. As a matter of fact the shaking out method is used only in four of the assay methods. In three of these the acid chosen is hydrochloric, and it is noticeable that in each of these cases the results are low. In the one case where sulphuric acid is used, results are normal. As has been already pointed out (50) the alkaloid strychnine can be completely extracted from a solution containing excess of free hydrochloric acid by shaking out with chloroform.

**358. Other assay methods** more or less in use. Dunstan and Short direct to extract 5 gm. of powdered drug in a soxhlet apparatus one to two hours with a mixture of 40 mls of chloroform and 10 mls of alcohol and shake out the alkaloids in the usual manner. Bird directs to triturate 5 gm. of the powder with 2 mls of a 10 percent solution of potassium hydroxide, and exhaust by extracting with a mixture of 4 volumes of ether, 3 of chloroform and 1 of amylic alcohol. The Belgian pharmacopoeia employs the following rather crude method. Triturate 15 gm. of the powder with some fine sand and exhaust the mixture with 70 percent alcohol. Evaporate the solution to 10 gm., acidify with acetic acid and shake out with ether which is to be rejected. Add 50 gm. of ether, 25 gm. of chloroform, and 5 gm. of water of

10 percent nitric acid, the precipitate is collected on a quantitative filter, washed with water until free from acid, dried, ignited and weighed, the residue having the composition  $(\text{Wo O}_2)_{12} \text{Si O}_2$ . Multiply weight of this residue by 0.4984 to find weight of combined strychnine and brucine in the sample. (The factor is as uncertain as in the ordinary method of alkalimetric titration. Query, is brucine unaffected by nitric acid of the strength prescribed?)

**360. Assay by Picrolonic Acid.** The alkaloids of nux vomica form insoluble picrolonates of definite composition. H. Mather and O. Rammstedt† base on this fact the following assay process. Shake 10 gm. of the powdered drug with 100 gm. of ether, and 50 gm. of chloroform, add 10 mls of 10 percent solution of sodium hydroxide and shake for 10 minutes. Add 15 mls of water to agglomerate the drug, shake and let stand 20-30 minutes. Evaporate 75 gm. of the clear solution to one half, add 5 mls of a tenth-normal alcoholic solution of picrolonic acid and let stand 24 hours in a cool place. Collect the precipitate in a Gooch crucible, wash with 2 mls of alcohol-ether, 1 : 3, dry 30 minutes at  $110^\circ \text{C.}$ , cool in a desiccator and weigh. Multiply by 0.5798 to find the weight of combined brucine and strychnine in 5 gm. of the sample. (If proportion of strychnine is 40 percent the factor would be 0.5844. The best plan would be to use two equal aliquot portions of the alkaloidal solution, treating one with nitric acid to destroy brucine, precipitating both as above and using the factor 0.5586 to determine strychnine. The difference in weight between the two precipitates would be the weight of the brucine picrolonate, to be multiplied by 0.5988 for weight of brucine.)

**361. Assay of Galenical Preparations** of nux vomica presents no special difficulties. For the fluidextract follow type process (A) (110) using 10 mls of the fluid with chloroform as the immiscible solvent. In titration of the alkaloids use 10 mls of

\*Schweiz. Wschr. f. Chem. u. Pharm., 1913, 761.

†Arch. Pharm., 1907 (245), 112-32.

tenth-normal hydrochloric acid, with cochineal or ammonia, shake well for several minutes, let stand one hour. Decant 50 gm. of the ethereal solution, distil off the solvent, add to the residue 10 gm. of ether, evaporate on the water bath to constant weight and weigh.

**359. Assay by Silicotungstic acid.** A. Azadian \* extracts the alkaloids from 10 gm. of drug with ammonia and ether-chloroform 2:1 (weight). After distilling off the greater part of the solvent, he extracts the alkaloids by shaking out with 10 percent nitric acid. The alkaloids are precipitated with 10 mls of 5 percent silicotungstic acid, followed by 10 mls of methyl red as indicator. The alkaloid carries something like 15 percent of impurity, from which it may be freed in part by dissolving (before titration) in a minimum quantity of dilute acid, bringing to a definite volume with distilled water, filtering and extracting the alkaloids from an aliquot part of the solution. The alkaloids may be dried at 100° C. to constant weight and weighed, then dissolved in volumetric acid and titrated. The gravimetric result will probably still be high and the titration result a little low, so that the mean may be taken as a close approximation to the truth, but not nearer than that from titration in the usual manner.

**362. If a gravimetric determination is desired,** the best plan is to use type process B (111) which yields alkaloid so free from impurity that it dissolves to nearly a clear solution in dilute acid. Otherwise purify the alkaloid by use of petroleum ether (355), or by hard paraffin (353), the latter the expedient adopted in the assay of the British pharmacopoeia.

**363. Another method similar in principle** is that proposed by W. B. Cowie\*, as follows: Mix 10 mls of the fluidextract with 10 mls of diluted acetic acid (5 percent), evaporate off alcohol, cool to 15°C., make up to 45 mls with water and add 5 mls of the official solution of ferric chloride, shake well and filter. To 25 mls of the solution, corresponding with

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\*Pharm. Journ., 1914, 545.



5 mls of the fluidextract, add 15 mls of a 20 percent solution of sodium carbonate and extract the alkaloids by shaking out with chloroform. The alkaloids may be dried to constant weight and weighed, then dissolved and titrated in the usual manner. (The method is given for what it may be worth. One would expect better results from addition to the fluid extract of a sufficient quantity of tincture of ferric chloride making up with alcohol to 100 mls, adding 2 gm. of slaked lime, shaking well and filtering. An aliquot portion of the solution might be acidified with hydrochloric acid, evaporated to a small volume and the alkaloids extracted by shaking out with ammonia and chloroform.)

**364. Assay of extract.** Two gm. of the ordinary pilular extract may be dissolved in 10 mls of diluted alcohol and 2 mls of water of ammonia and the assay carried out as in the case of a fluidextract (110). The official extracts are now generally in powdered form, being standardized by the addition of various diluents—according to the U. S. P. IX, magnesia and dried starch, (British, calcium phosphate, French and Swiss, sugar of milk). The presence of insoluble diluents will embarrass the assay if made by the foregoing method. It is better to exhaust the powder with 70 percent alcohol, evaporate to a syrupy consistence and then treat as above.

**365. Alternative method** for a powdered extract. Dissolve 2 gm. of the sample as completely as possible in 10 mls of 70 percent (vol.) alcohol. Add 85 mls of alcohol, shake well, add 3 mls of solution of ferric chloride (U. S. P.) and make up with alcohol to 100 mls, add freshly slaked lime 1.5 gm. and shake well during 2 minutes. Filter, and to 50 mls of the filtrate, equivalent to 1 gm. of the sample, add 5 mls of tenth-normal hydrochloric acid and evaporate nearly to dryness. Dissolve the residue in 10 mls of hot water, transfer (with washings) to a small beaker and treat with 2 gm. of hard paraffin to remove fatty matter. Extract the alkaloids by shaking out with chloroform, after adding ammonia; evaporate to

dryness, dry the residue and weigh (as check on the titration results) then dissolve and titrate as usual.

366. **Determination of strychnine** in galenical preparations is to be made precisely as in assays of the crude drug (354).

367. **Strychnine and Quinine** may be quantitatively separated and determined, according to A. R. Bliss, by the following simple procedure\*. Extract the combined alkaloids (e. g. from an elixir) in the usual manner. Dissolve 0.5 gm. of the mixture in 5 mls of 5 percent sulphuric acid, transfer the solution to a separator by aid of distilled water sufficient to bring the volume to 250 mls (i. e. to 6.5 mls for each milligram of quinine). Render alkaline with water of ammonia and shake out with seven portions of ether (35, 20, 10, 10, 10, 5 mls), wash the ethereal solution with 5 mls of distilled water, transfer it, with the usual precautions, to a tared beaker, evaporate, dry one hour at 100° C. and weigh as quinine. Shake out the residual aqueous solution in the separator with seven portions of chloroform (35, 20, 10, 10, 10, 10, 5 mls). Wash the chloroform solution with 10 mls of distilled water, transfer to a tared beaker, evaporate, dry the residue one hour at 100° C. and weigh as strychnine. Results reported are good. The methods hitherto proposed—precipitation of the strychnine by potassium ferrocyanide or of the quinine as tartrate or oxalate—do not give accurate results.

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\*Jn. Am. Phar. Assoc., 1919, 804-7.

**OPIUM**

**368. The sedative or narcotic action of opium** is due chiefly to the morphine it contains. Of the associated alkaloids having a sedative action, the only one of any importance is codeine. Narcotine, which is present in considerable quantity, is tonic rather than sedative. In assays of the drug, it is not usually taken into account, and as a rule valuation of a sample of the drug is based solely on its morphine content.

**369. Morphimetric assay of Opium** is carried out commonly in one of the three following general methods: (1) the opium is treated with slaked lime and a comparatively small accurately measured quantity of water, whereby the morphine is brought into solution in combination with lime, leaving behind nearly all the other alkaloids; on addition of ammonium chloride to an aliquot part of the solution, the morphine is thrown out of solution in crystalline form; (2) the opium is extracted thoroughly with water, the solution concentrated and the morphine precipitated from it under certain prescribed conditions with water of ammonia; (3) the alkaloids of opium other than morphine are separated from an aqueous solution by shaking out with chloroform after adding potassium or sodium hydroxide, the solution is neutralized and then again made faintly alkaline with ammonia and the morphine extracted by shaking out with a mixture of chloroform and alcohol—or else with some other special immiscible solvent—and determined by alkalimetric titration.

**370. Of the lime method** it may be stated that: (1) it yields morphine practically free from impurities, the initial step in the assay affecting a sharp separation between morphine and the other alkaloids of opium. (2) It is the most expeditious method yet devised; possibly the shaking out process may be completed in a shorter time, but the latter calls for the undivided attention of the operator, while the former consumes very little of the operator's time. (3) It eliminates

largely the personal equation of the operator. This is partly because it requires a minimum of manipulative skill, partly because the residual morphine is a remarkably small as well as a very constant quantity.

**371. Per Contra:** (1) The lime assay involves unavoidably the principle of the aliquot part, the exactness of the aliquot difficult to insure. (2) Crystallization of the morphine is from a comparatively dilute solution, but this objection is offset by the absence from the solution of impurities hindering the process of crystallation and frequently contaminating the product. (3) To obtain by it rigidly exact results, a correction for solubility of morphine must be made. The same however is true in the case of the second general assay method, in which there is greater uncertainty about the corrective constant. The most serious objection is that for reasons not yet fully understood, the lime process applied direct to the drug fails in many cases to extract the whole of the morphine. Until we ascertain the reason for this, we are obliged to exhaust the opium in the outset with water and apply the lime assay to this aqueous extract. This lengthens the time required for the assay and at the same time introduces an element of uncertainty since opium is known to contain morphine not extracted by water. (This difficulty is met with also in assays by the second general method.)

**372. All things considered,** the lime assay is to-day that best suited to the needs of pharmacist and physician, and quite worthy of confidence as a means of determining the comparative commercial and medicinal value of different samples of opium. The assay must of course be carried out rigidly in accordance with each detail in the prescribed method. Whether some of the newer shaking out methods will prove to be more exact and trustworthy remains to be seen.

**373. The second general method** has its strong advocates. Under its most approved modifications, it yields results satisfactorily concordant, the impurities contained in the morphine being

generally removable without much trouble. Insoluble morphine may probably be extracted by judicious use of a small quantity of oxalic or tartaric acid, or else the marc can be treated with lime and the residual morphine thus determined. The morphine obtained by direct precipitation from a concentrated aqueous solution generally contains codeine, which may be extracted by treating the crude alkaloid with caustic alkali and shaking out with chloroform, the morphine remaining in solution.

**374. The impurity** which gives most trouble is calcium meconate; this and perhaps other calcium salts may be excluded by the use of alcohol as herein-after described (386), or a little ammonium oxalate may be used (392) to throw down calcium.

**375. The new shaking out processes** promise well although they are rather tedious. As yet it remains to be proved whether they effect complete isolation and extraction of morphine. They are undergoing thorough study and some of them are likely to come into general acceptance, and ultimately to furnish the basis for international standards for opium and its preparations.

**376. Sampling of the opium**, if in its original crude form, is an important part of the assay. The drug comes in the form of balls or cakes weighing from 50 gm. to a kilogram, the interior generally more or less moist while the outer portion is, at least superficially, hard and dry. Inasmuch as the several cakes in a package are liable to vary much in morphine strength, it is customary to take from one in every five, large and small, a cone shaped piece, the apex of the cone as nearly as possible the center of the cake, and the sample judged to be a certain fraction, e. g. one tenth or one twentieth of the whole. These several pieces are kneaded together or pounded in a mortar. When the opium is reasonably soft, the sample may be brought into a homogeneous condition, as suggested by L. H. Bernegau, by running it through a meat grinder a few times. If quite dry and hard, it must be reduced to a powder. To effect

this, it is generally necessary to dry it in a current of warm air, making note of the loss in weight. Overheating in the drying must of course be avoided.

**377. Routine of the lime assay.** The U. S. P. IX employs for opium and its several preparations a lime assay process, using in each case 50 mls of an aqueous solution, representing uniformly the equivalent of 8 gm. of opium. When opium itself is assayed, the drug is exhausted with water precisely as described in (384) the volume of the solution from 8 gm. of drug being made up to 50 mls.\* The method is an adaptation of Dr. A. B. Stevens' very neat assay process†. Place in a small mortar 4 gm. of freshly slaked lime (not quick lime as prescribed in Stevens' original process). Add a few mls of the opium solution and rub to a smooth paste, add gradually the remainder of the solution with constant trituration, rinse the flask with exactly 10 mls of distilled water and add this to the lime mixture, which is to be stirred frequently during 15 minutes, avoiding appreciable loss of water by evaporation. Filter through a dry filter (the filtration is facilitated by previous straining through muslin) and transfer exactly 30 mls of the filtrate to a 100 ml Erlenmeyer flask. Add immediately 2 mls of alcohol and 15 mls of ether, shake and add 1 gm. (better 0.5 gm. Ed.) of ammonium chloride, stopper the flask, shake frequently during half an hour, then set aside in a cool place 12 hours or over night. Remove the stopper and brush any adhering crystals into the flask. Decant the ether into a small funnel the neck of which is plugged with purified cotton. Add to the flask 15 mls of ether, rotate and, when separated, decant this also into the funnel. When this has drained off, wash funnel and cotton with 5 mls of fresh ether and then pour the aqueous liquid also into

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\*Experience has shown that the yield of morphine is greater in many cases when the opium is thus first extracted with water, hence the adoption of this as the official assay method. If the Asher assay (379) secures as complete exhaustion of the opium it should surely be given preference to the official method.

†Proc. Am. Pharm. Assoc., 1902, 863; 1903, 786.

the funnel, carrying with it a portion of the crystals. Rinse the flask with successive small portions of water saturated with morphine, as long as they take up color, passing them also through the funnel. Wash funnel and cotton with one or two mls of water, transfer the cotton with the crystals it has retained to the flask. Rinse into the flask any crystals that have adhered to the funnel, add 20 mls of tenth-normal hydrochloric acid and when the morphine is completely dissolved, titrate the excess of acid with lime water (70).

**378. The aliquot taken** (30 mls) is really too large; 29.5 would be theoretically nearly right. If it were intended to make the aliquot large enough to offset the solubility of morphine in the mother liquor, 30.5 mls should be taken. Ordinarily in assays by the lime method an arbitrary correction figure is prescribed, amounting often to as much as 1 percent of the weight of the opium, so that results by the lime assay are as a rule too high. It is better to make the aliquot as nearly as possible correct. (An exact invariable figure cannot be stated, since the proportion of extractive in different samples of opium, and the ratio of morphine to extractive, vary greatly.) If the correct aliquot is taken, an arbitrary correction, perhaps of 0.5, should be added to the percentage of morphine. If no alcohol is added to the lime solution, the correction is smaller—perhaps near 0.3 percent.

**379. The lime assay applied to the drug itself** may be carried out in the following manner, as prescribed by Dr. Philip Asher\*,—a modification of Stevens' assay†; Place 4 gm. of granulated or powdered opium in a tared porcelain dish, add 5 mls of a 5 percent solution of potassium hydroxide or the equivalent of a stronger solution, mix thoroughly and evaporate on a water bath or in a drying closet to constant weight. Add 2 gm. of freshly slaked lime and exactly 10 mls of water. Triturate (15 minutes)

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\*Am. Journ. Pharm., 1906, 262-7.

†Proc. Am. Pharm. Assoc., 1902, 862; 1903, 786.

to a smooth paste, add exactly 19 mls of water and triturate frequently during half an hour and filter through a dry filter (10 cm.). (In foregoing operations guard against any loss by evaporation.) Transfer exactly 15 mls of the solution to a 100 ml Erlenmeyer flask and add to this 4 mls of alcohol (not more than 2 mls, and better none at all. Ed.) and 15 mls of ether. From this point carry out the assay exactly as in (377).

**380. Moist opium** may be assayed in the same manner, being previously dried and reduced to a No. 25 powder (finer if practicable), the loss in weight in drying being taken into account in calculating the result. The opium may be used without drying. The water in the moist sample having been determined, an equal amount must be deducted from the quantity of water used. The opium in small pieces is placed in a well stoppered flask with 10 mls of water and digested at a temperature of about 50° C. with frequent shaking until completely disintegrated. Cool the mixture, add 2 gm. of slaked lime, incorporate well, add the remainder of the water, viz. 19 mls minus the quantity already present in the sample, macerate with occasional or continuous shaking at least an hour, then proceed with the assay as in (397). The use of potassium hydroxide as prescribed by Dr. Asher may or may not be important. At all events complete extraction of the morphine can be effected with lime alone provided the mixture is shaken actively during at least two hours. Experiments should be made to determine the velocity of the reaction under prescribed conditions. However, we may safely adhere to the plan of the U. S. Pharmacopœia, modifying the procedure perhaps by omitting a part or the whole of the alcohol, and certainly by correcting the result for solubility of morphine (378).

**381. Modified lime assay of G. Guerin.\*** Powdered Opium 7.5 gm., slaked lime 3 gm., water 30 mls to form a magma, then 45 mls, by aid of which the magma is transferred to a stoppered flask

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\*Journ. Pharm. Chim., 1913, 162.



in which it is left 2 hours with occasional gentle agitation. Of the filtered solution 52 mls is taken as an aliquot equivalent to 5 gm. of drug (the true aliquot would not greatly exceed 51 mls). Five mls of acetone are then added, followed by 1 gm. of ammonium chloride, the mixture is set by 24 hours to crystallize, the crystals are collected on a pair of counterpoised filters, washed with morphinated water until free from chlorides, then with 4 successive portions (15 mls) of anhydrous acetone saturated with morphine, and finally dried at 100° C. and weighed. No correction is made for dissolved morphine. (It should be determined experimentally whether such correction is needed).

**382. Assay by Barium Hydroxide.** J. Perger directs:\* Boil 10 gm. of the opium a short time with 150 mls of water and 15 gm. of barium hydroxide. Filter and boil the residue repeatedly with small quantities of water until it fails to give a reaction with sulpho-molybdic acid. Excessive boiling is to be avoided, and the volume of the filtrate should not be above 400 mls. Evaporate rapidly on the water bath to dryness. Moisten the residue with dehydrated alcohol, transfer to an Erlenmeyer flask and exhaust by repeatedly boiling with dehydrated alcohol, of which 200 to 300 mls will be required. Distil off the alcohol, add 15 mls of water containing some ammonia and allow to stand sometime. Stir well with a glass rod, collect the morphine on a tared filter dry at 40° C., and treat repeatedly with chloroform. Weigh as hydrated morphine and confirm by titration. Multiply by 0.9406 to find the weight of anhydrous morphine.

**383. New Assay Process of A. Tingle.†** The U. S. P. IX assay ignores the water-insoluble morphine in opium.‡ Dr. Tingle, after an exhaustive study of the problem of opium assay, proposes the following as applicable not only to all forms of opium, including

\*Journ. pr. Chimie, (2) 29, 97, 110; Journ. Chem. Soc., Nov. 1884, 1217.

†Am. Jr. Pharm., 1918, 854-6.

‡I. Debourdeaux in Bull. Sci. Pharmacol., 1910, 382-5.

those containing water-insoluble morphine, but also to complex mixtures in general containing morphine. Digest 6 gm. of the sample in a 100 mil measuring flask 10 minutes on a water bath with 2 gm. of calcium carbonate mixed with water enough to form a thin paste. Cool and add 60 mils of a cold-saturated solution of barium hydroxide. Stopper the flask and shake well at frequent intervals during 25 minutes. Add water to make up 100 mils. (No allowance is made for the volume of the calcium carbonate and that of the opium marc, a matter obviously of considerable importance. The error in aliquot may be taken to offset the insolubility of morphine in water, but compensations of this kind ought to be brought to an exact basis, which would require exact measurement or weighing of the water used—also of the aliquot and the extract contained in the aliquot). Filter the solution through a dry filter. Transfer 50 mils of it to a 55 mil measuring flask, add sulphuric acid, (diluted 1:5) drop by drop to faint acidity, warming the flask to aid precipitation. Add cautiously a strong solution of sodium hydroxide to faint alkalinity, then salicylic acid in crystals to restore acidity and finally 0.5 gm. more of the acid. Heat 10 minutes in a boiling water-bath, cool, and make up to 55 mils, at room temperature. Filter through a dry 7 cm. filter paper.

384. Transfer 50 mils of the solution to a platinum dish and evaporate nearly to dryness on the water-bath. Cool the dish and add water sufficient to make the volume 5 mils. Add 5 mils of chloroform and 3 drops of stronger water of ammonia or sufficient to render the solution sharply alkaline. Mix the fluids with a stirring rod which is allowed to rub the bottom of the dish to promote crystallization. When crystals appear, cover the dish and set it aside 4 hours, occasionally agitating it with a rotary motion. Filter through a plug of absorbent cotton with aid of suction, washing the dish and the crystals in the funnel with a minimum quantity of water, which may be morphinated (10 mils). Dissolve crystals remaining in the dish and those in the funnel in a little diluted

sulphuric acid, follow with water to bring the solution to 20 mls. Transfer the solution to a separator, make just distinctly alkaline with ammonia, adding 1 drop of 10 percent ammonia in excess, and shake out with 4 successive portions (30 mls) of a mixture of chloroform and alcohol (2 : 1 vol.), shaking 3 minutes each time (399). Evaporate the solvent, dissolve the residue in 20 mls of tenth-normal acid, and determine morphine by residual titration as usual (70). Multiply the quantity of morphine in grammes by 36.67 to find the percentage of morphine in the sample.

**385. Routine of Assay by Aqueous Extraction.**

The opium may be powdered or granulated or it may be in the moist condition. (In every case a sample should be dried to constant weight and the loss of weight noted, so that results can be calculated to the basis of dry drug.) Macerate in a covered beaker 8 gm. of the sample, preferably over night, with 80 mls of distilled water at a temperature of 50° C. Otherwise macerate (at that temperature) with frequent shaking 3 hours or more, until the drug is completely disintegrated. Filter the solution through a 12 cm. filter, bringing the marc on to the filter by aid of portions of the filtrate. Wash the residue on the filter with distilled water sufficient to bring the volume of the filtrate to 120 mls. When fully drained, return the residue carefully to the beaker, mix to a smooth cream with distilled water, let stand 15 minutes then bring it again upon the same filter. Wash the residue with 75 mls more of distilled water. If necessary treat the marc once more as above to secure complete exhaustion. To test the necessity of this, collect 50 mls more of filtrate, evaporate to about 1 mil, add to this in a test tube 2 mls of ether and just enough ammonia to produce distinct alkalinity. Stir with a glass rod and shake for a minute or more, and observe whether crystals of morphine appear.

**386.** Evaporate the mixed filtrates in a flat dish over a water-bath to a volume of 20 mls, add 70 mls of alcohol, let stand 20 minutes with occasional stirring then filter through a 12 cm. filter; wash the precipitate and filter with several portions of a mixture

of alcohol 3 volumes, water 1 volume\*. Evaporate the mixed filtrates to a syrupy consistence, dissolve in 8 mls of distilled water and transfer to a tared 50 ml Erlenmeyer flask. Rinse the dish with several small portions of distilled water, bringing the weight of the solution to 20 gm. Add alcohol 12 mls, shake, add ether 25 mls, shake, add water of ammonia (10 percent) 3 mls. Cork the flask and shake continuously ten minutes, then shake at frequent intervals during half an hour, after which set the flask aside in a moderately cool place 12 to 18 hours.

387. Decant the ether into a small beaker and thence pour it upon a pair of mutually counterpoised 7 cm. filters. Add to the flask 15 mls of fresh ether, rotate and when separated decant as before into the beaker and thence onto the filter. Repeat with 2 or 3 fresh portions of ether (until this is no longer colored). When the ether has passed through the filter, pour on the mixture containing the morphine crystals, transferring the last of these to the filter by aid of portions of the filtrate. Wash the crystals on the filter with a saturated solution of morphine in distilled water until this passes colorless, then wash the crystals with 5 mls of distilled water. When drained, press the filters between folds of filter paper, dry at a temperature not above 50° C. to constant weight and weigh.

388. **Determine the purity of the alkaloid** by the following method.† Rub the crystals to powder in a small mortar, treat 0.250 gm. with 25 mls of boiling absolute alcohol (neutral), as long as anything is dissolved. (As a rule there will be almost no residue.) Filter and wash residue on the filter with hot absolute alcohol until free from alkaloid. Dilute filtrate and washings with an equal volume of distilled water (neutral) and titrate with tenth-normal acid, using methyl red as indicator (titration factor 0.0285), evaporate off alcohol from the titrated solution on a water bath, add 10 mls of tenth-normal

\*Compare the procedure of R. Lamar, Am. Journ. Pharm., 1900, 36-9.

†C. E. Smith in Am. Journ. Pharm., 1916, 292.

potassium hydroxide and shake out the alkaline solution with five portions (10 mls) of chloroform, which will take out alkaloids other than morphine (more particularly codeine). Evaporate off the chloroform and titrate the residue calculating it as morphine, to be deducted from the result of the first titration. [The alkaloidal impurity may be calculated also as codeine (factor 0.0299)]. Finally a correction for solubility of morphine ought rightfully to be made, amounting, according to R. N. Shreve\*, for each precipitation by foregoing method, to 18 mg. at 17° C., and 26 mg. at 29° C. A uniform correction of 25 mg. would seem to be reasonable.

389. **A simpler and equally efficacious plan** is to use instead of the dehydrated alcohol, 30 mls, accurately measured, of lime water, the normality of which has been determined. The morphine (0.300 gm.) is shaken with this until no more is dissolved, then the solution is left at rest until the upper portion is perfectly clear. Ten mls of this clear solution equivalent to 100 mg. of the crude alkaloid are titrated with volumetric tenth-normal hydrochloric acid. The increase in alkalinity is due to dissolved alkaloid, which is calculated as morphine. Add to the titrated solution 1 ml of normal potassium hydroxide and shake out with 5 portions of chloroform (10 mls) to extract alkaloids other than morphine (codeine). See (388).

390. Mallinckrodt determines the **purity of crude morphine by a reassay** (by the lime method) as follows: Mix in an Erlenmeyer flask 1 gm. of the crude morphine with 0.45 gm. of slaked lime and 18 mls of water, shake occasionally during one hour. Filter into a tared 60 ml Erlenmeyer flask, with gentle suction; wash flask and residue with lime water until total filtrate amounts to 30 gm. Add 2.5 mls of alcohol, rotate, add 17 mls of ether, rotate, add 0.45 gm. ammonium chloride, cork and shake vigorously. Determine morphine as in (388), adding 0.030 gm. for solubility of morphine in the mother liquor. (No provision is made for detection or determination of codeine in this procedure.)

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\*Journ. Ind. and Eng. Chem., 1912, 517.

**391. Extraction of the opium with hot water** is practised by some (method of J. Howard Wainwright\*). The objection made to this is that the hot water extracts much narcotine, which is almost insoluble in cold water. The narcotine however is held in solution by the ether used in the crystallization process. The use of warm water in the initial maceration is not objectionable. When the opium is in powdered or granulated form, a preliminary extraction with ether or with petroleum benzin leaves the drug practically free from narcotine, so that hot water may be used preferably for exhausting the drug.

**392. Instead of using alcohol** as directed in (386), we may add to the water used in the first maceration of the opium 0.25 gm. of ammonium oxalate, by which calcium salts are excluded from the solution. The result is not quite so satisfactory. It is possible, of course, to use both the oxalate and alcohol, but with questionable advantage.

**393.** It remains to consider the possibility that a **part of the morphine** may exist in an **insoluble** condition. It seems probable that such morphine could be extracted by treating the marc with water containing a little free acid—perhaps best hydrochloric. The solution could be nearly neutralized before concentration but it would not be advisable to add it to the main solution. It might preferably be treated separately for extraction of the morphine; the best procedure can be determined only by trial. One may suggest evaporation to dryness with a little calcium carbonate, extraction of the residue with dehydrated alcohol (neutral) and titration with volumetric acid, with subsequent extraction of alkaloids other than morphine as in (388).

**394. The Dieterich Assay** (modified), as official in the German pharmacopœia. Seven gm. of powdered opium are mixed with 7 gm. of water and 49 gm. of water are gradually added. The mixture is shaken frequently during one hour, filtered and to

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\*Journ. Am. Chem. Soc., VII, p. 48.

42 gm. of the filtrate are added 2 mls of a mixture of 17 gm. of water of ammonia (10 percent) with 83 gm. of water, avoiding active shaking, and the mixture is immediately filtered through a dry filter. 36 gm. of the filtrate, representing 4 gm. of opium, are shaken with 10 mls of acetic ether, 5 mls of the above ammonia solution are added and the mixture is shaken 10 minutes; 20 mls of acetic ether are added and the mixture is shaken occasionally during 15 minutes. The acetic ether is poured on to a filter, followed by the aqueous solution, the flask containing adhering crystals of morphine is rinsed three times with ether (5 mls) which is used also to wash the filter. Filter and flask are dried at 100° C., the crystals from both are dissolved in 25 mls of tenth-normal hydrochloric acid and the excess of acid is titrated with tenth-normal potassium hydroxide. The method is suited for rapid approximate determinations of morphine, but lacks the exactness of the methods of (385-388).

**395. Lead subacetate method of C. E. Parker\*.** Shake 10 gm. of opium with 100 mls of water 2½ hours by aid of a mechanical shaker, add 25 mls of solution of lead subacetate, shake ½ hour. Filter through a wetted filter and wash the residue with water to make up 175 mls of filtrate. Mix the marc with 50 mls of water, shake 10 minutes and return to the filter. Wash residue with water to make up 150 mls of filtrate. Unite the filtrates and add from a burette normal oxalic acid solution, at first 5 mls at a time, stirring and allowing to settle after each addition, afterwards 2 and 1 ml at a time until a precipitate is no longer produced (in all about 26 mls), then add 5 mls more. Filter, wash the precipitate with water, evaporate in flat bottomed dishes to volume of about 20 mls. Precipitate lead in the concentrated solution with hydrogen sulphide, filter through a 4 cm. filter, wash with a minimum quantity of hot water. Evaporate to a volume of about 10 mls, transfer to a tared 100 ml flask, rinsing with small portions of water to bring the weight to 20 gm. Add 12 mls of alcohol

\*Proc. Am. Pharm. Assoc., 1907, 490-5.

and 25 mls of ether, rotate and add 2 mls (or a slight excess) of water of ammonia, cork the flask, shake and suspend under the cork a strip of neutral litmus paper, which should become blue in about one minute. The crystals are to be treated as in (388).

**396. Separation of Opium Alkaloids from one another.** The following scheme\* for separation of the principal alkaloids of opium may contain suggestions useful to some. To an aqueous solution containing narcotine, papaverine, narceine, thebaine, codeine and morphine, add a concentrated solution of sodium acetate and let stand 24 hours. Narcotine and papaverine are precipitated. Dissolve the washed precipitate in dilute hydrochloric acid and dilute to 400 times its weight, add potassium ferrocyanide to precipitate (in 24 hours) papaverine. From the filtrate narcotine is precipitated by neutralizing with ammonia. Concentrate the solution from which narcotine and papaverine have been removed and let stand 24 hours. Narcotine will separate. Filter and add sodium salicylate. After 24 hours, filter out thebaine salicylate. Acidulate the filtrate with hydrochloric acid and shake out repeatedly with chloroform to remove remaining salicylic acid, narcotine and thebaine. Drive off dissolved chloroform by a gentle heat and add potassium sulphocyanate to precipitate codeine. After 24 hours, filter and to the filtrate add ammonia to precipitate morphine.

**397. Assay by extraction with Sodium Chloride solution.** C. Montemartini and D. Traschiatti after testing many methods for the assay of opium approve those of Perger and of Dr. Squibb—essentially that of (384-387)—but offer the following as equally good†. Macerate 10 gm. of the powdered opium in a mortar with 90 mls of a 20 percent solution of sodium chloride for one hour and filter. Treat the residue one hour with 60 mls of the salt solution, filter and percolate the residue further with the same solution until a drop gives no reaction with Froehde's

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\*P. C. Plugge, Arch. de Pharm. 1887, 393.

†Gazetta Chim. Ital., 27, II, 302; Journ. Chem. Soc., 1898, A, II 270-1.



reagent\*. Evaporate the mixed solutions to dryness on the water-bath, extract the powdered residue with boiling absolute alcohol (300-350 mls) until the fluid ceases to give a reaction with Froehde's reagent. Evaporate or distil, cover the residue with 15 mls of a very dilute solution of ammonia and let stand 24 hours. Collect the separated morphine on a tared filter, wash with morphinated water until the washings are colorless, and dry at 100° C. Wash the crystals well with chloroform, dry at 100° C. and weigh.

**398. Assay by shaking out** with alcohol-chloroform. If an opium solution containing calcium hydroxide is shaken with chloroform repeatedly, the opium alkaloids with exception of morphine will be completely extracted. If then the aqueous solution be acidulated with hydrochloric acid and then made slightly alkaline with ammonia, the morphine can be extracted by repeated shaking out with chloroform containing alcohol. The following details no doubt admit of modification, but if carefully followed they make possible a scientific and reasonably satisfactory determination of morphine in opium.

**399.** Put into a suitable flask 1 gm. of powdered opium, add 100 mls of lime water, stopper and shake well every 10 minutes during two hours. Allow to settle and filter rapidly through a dry plaited filter. Transfer 50 mls of the filtrate at once to a separator (No. 1). Shake out seven times with chloroform (30 mls) previously washed with water. Collect the chloroform in separator No. 2. Shake out the liquid in No. 1, with 30 mls more of chloroform, collect this in separator No. 3 containing 15 mls of clear lime water. Shake, let separate, draw off the chloroform, filter if necessary and evaporate to dryness. Dissolve the residue in a few drops of water acidulated with hydrochloric acid, and test with Mayer's reagent. If any precipitate is produced repeat the shaking out of the original solution with chloroform, which is

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\*In place of Froehde's reagent, use a mixture of sulphuric acid (conc.) 1 mil and solution of formaldehyde (30 percent) 1 drop, applied to the residue after evaporating the drop of weak percolate to dryness.

washed with the lime water in No. 3 and tested with Mayer's reagent as before. Repeat this procedure as long as Mayer's reagent produces any cloudiness.

400. Now add the lime water in No. 3 to the chloroform in No. 2, shake well and reject the chloroform. Wash the lime water with 30 mls of fresh chloroform, reject this and add the lime water to No. 1, which contains now no alkaloid except morphine. Carefully neutralize the solution with dilute hydrochloric acid, add then water of ammonia three drops, and shake out until completely extracted with a mixture of chloroform 2 volumes and alcohol 1 volume, of which 30 mls are used for each extraction. Not less than 7 portions of the chloroform-alcohol will be required—probably 10 or more. There is always a tendency to the formation of emulsions, so that the shaking out must be done with due restraint. Generally it will be best to draw off into a separator (No. 4) the last, partially emulsified portion of the chloroform and shake this with the next portion of chloroform-alcohol, before this is added to No. 1. It is an advantage to add to the solution in No. 1 sufficient sodium chloride to produce a half-saturated solution. This will perhaps increase the tendency to emulsification, but it will make extraction of the alkaloid more rapid.

401. Finally evaporate the united chloroform solutions (after washing with water, and in turn washing the water with chloroform-alcohol) on a water-bath, dissolve the residue in hot neutral alcohol add 5 mls of tenth-normal hydrochloric acid, dilute with an equal volume of neutral alcohol and titrate excess of acid with lime water, using methyl red as indicator.

**402. Improved process of Dr. Dohme\*, based on experiments of Anneler†.** A solution is prepared

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\*Journ. Am. Pharm. Assoc., 1915, 85-92.

†Arch. d. Pharm., CCL, 187. See also paper by J. B. Nagelvoort on the use of isobutyl alcohol for the detection of morphine and codeine; Proc. Am. Pharm. Assoc., 1894, 273-7.

by aqueous extraction of which 50 mls represents 4 gm. of opium. The solution is made decidedly alkaline with sodium or potassium hydroxide and shaken out with several portions (20 mls) of ether to remove alkaloids other than morphine. Dilute sulphuric acid is then added to faint acid reaction, then water of ammonia in slight excess, and the morphine is extracted by shaking out repeatedly with a mixture of equal volumes of chloroform and isobutyl alcohol. The solvent is distilled off—the chloroform under ordinary conditions, the isobutyl alcohol under reduced pressure, the residue is taken up with an excess of volumetric acid, and the morphine determined by residual titration in the usual manner.

403. **Phenylethyl alcohol** for shaking out morphine. One of the most active solvents known for morphine is phenylethyl alcohol. A. D. Thorburn \* gives details of a method of using this solvent in the determination of morphine, particularly in tablets, pills, etc. When used by itself it produces troublesome emulsions, but if mixed with one-third its volume of benzene, it does not show this tendency, provided the morphine solution contains about 30 percent (vol.) of ethyl alcohol. A solution is made containing in 15 mls not more than 0.175 gm. of morphine. The solution if acid is neutralized with water of ammonia, 4 drops more of concentrated ammonia solution are added, and the alkaloid is extracted by shaking vigorously with a mixture of 3 mls of phenylethyl alcohol and 1 ml of benzene (The shaking must be prolonged several minutes). A second and third extraction must be made, using one-half the foregoing quantities of solvent. Heat the mixed solutions on a boiling water-bath one hour, transfer the residue to a separator by aid of several portions of ether (20 mls in all), add 10 mls of tenth-normal sulphuric acid, shake vigorously 5 minutes, and at intervals during half an hour. Draw off the separated acid solution, wash the residue 3 times with water (3 mls)

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\*Journ. Ind. and Eng. Chem., 1911, 754-6.

shaking well together for several minutes. Finally titrate the excess of acid in the mixed solutions, as usual.

**404. Galenical preparations of opium** may be assayed by either of the foregoing general methods, having first prepared an aqueous solution of the prescribed strength. Alcoholic preparations as a rule throw down much narcotine and resinous matter when diluted with water. Such a precipitate is to be separated by filtration and rejected, the filtrate being concentrated by evaporation to a strength corresponding with that prescribed in the assay process to be used. In the assay of extract of opium by the process of (386-388), bear in mind the possibility that sugar of milk may have been used to standardize the product, in which case it may happen that the morphine is contaminated with milk sugar. The discrepancy between the results of titration and of weighing the alkaloid will show such contamination.

## PHYSOSTIGMA

**405. Standardization of physostigma** and its galenical preparations is based on their content of ether-soluble alkaloid. Owing to the instability of the alkaloid, it is questionable whether such standardization insures uniformity in therapeutic efficiency. The drug is practically not used in substance. The fluidextract is probably more worthy of confidence than any other galenical preparation although this is not official in U. S. P. IX. It is likely that hereafter the salts of physostigmine (eserine) and possibly of other alkaloids present in the drug will come to take the place of galenical preparations.

**406. Assay of the drug** may be made by type process I (102), using ether alone for the immiscible solvent and an alkali bicarbonate to set free the alkaloid. The process of H. Beckurts\* is perhaps the best. He directs to use 20 gm. of the drug in fine powder and 120 gm. of ether (200 mls is better)

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\*Apoth. Ztg., 1905, 670.

with 10 mls of a 10 percent solution of potassium bicarbonate (alkali carbonates and sodium bicarbonate should not be used). The mixture is shaken frequently during 3 hours. An aliquot of the ether representing 15 gm. of the drug is taken for the assay, one half the ether is distilled off and the residue, after addition of 10 mls of petroleum benzin, is shaken out with tenth-normal hydrochloric acid (10, 5, 5 and 5 mls.) To the united acid solutions is added 45 gm. of ether and 10 mls of a 10 percent solution of potassium bicarbonate, the mixture is well shaken and allowed to separate. An aliquot of the ether is treated with 10 mls of hundredth-normal hydrochloric acid, 20 mls of water and 5 drops of iodeosin indicator and the excess of acid titrated with hundredth-normal alkali. Each mil of standard acid consumed corresponds with 0.002752 gm. of alkaloid estimated as physostigmine. Under the conditions of the assay, calabarine, being insoluble in ether, is excluded, while the other alkaloids are not materially affected by the alkali used.

**407. Assay process of U. S. P. IX.** The quantity of drug taken is only 15 gm. Not less than 20 gm. of drug should be used and 25 is better. On account of the strong tendency to emulsification, the aliquot of the primary immiscible solvent (ether) is evaporated by exposure to a current of warm air after addition of 20 mls of tenth-normal sulphuric acid, the acid solution is decanted through a filter into a separator, the fatty and resinous matter which separates is redissolved in 15 mls of ether and the solution treated with 2 mls of tenth-normal sulphuric acid, evaporated as before and the acid solution added to that in the separator. The filter is washed with water, sodium bicarbonate is added to alkalinity, and the alkaloid extracted by shaking out with ether. The ether is evaporated, the alkaloidal residue dissolved in 2 mls of tenth-normal acid and determined by residual titration in the usual manner. Great care must be taken not to expose the alkaloid to heat over 65° C. or even to strong light. It will be safe

to substitute potassium bicarbonate for the sodium salt prescribed.

**408. Assay of the fluidextract or tincture.** To avoid emulsification, the U. S. P. IX resorts to the sawdust expedient (112), and it is perhaps as well to follow this general method, remembering that cheese cloth (113) answers the same purpose as purified sawdust. However, the lead subacetate method (111) is simpler and much more rapid. It is equally applicable to the extract, and its principle can be used advantageously in the assay of the drug itself after evaporating off the primary solvent.

**409. Extract (powdered) of physostigma** (3 gm.) is simply mixed with sand (10 gm.) and assayed in the same manner as the drug (407). Possibly solid paraffin may be used to separate fatty matters. See (293).

## PILOCARPUS

**410. For purposes of standardization,** determination is made simply of the total alkaloids contained in the leaves. Assay of the leaves is made by type process I (102), using for the primary immiscible solvent chloroform instead of a mixture of ether and chloroform, following otherwise the detail of the process for assaying belladonna leaves. Each mil of tenth-normal volumetric acid corresponds with 0.020815 gm. of alkaloid estimated as pilocarpine. The U. S. P. IX requires that the leaves contain not less than 0.6 percent of alkaloid, and drug of this high standard now comes to the American market.

**411. Assay by Picrolonic Acid** according to H. Matthes and O. Ramstedt\*. Digest 15 gm. of the sample in medium fine powder with 150 gm. of chloroform and 15 gm. of water of ammonia for 30 minutes with shaking, throw the mixture upon a large plain filter and cover with a clock glass. When filtration slows up, start it again by pouring on some water. Having collected something over 100 gm. of chloroform, shake it vigorously in a flask with 1 mil

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\*Arch. Pharm., 245, 112-32.

of water and set aside 1 hour to clear. Evaporate 100 gm. of the clear chloroform solution to about 20 mls, mix with 3 mls of tenth-normal alcoholic solution of picrolonic acid (0.02641 gm. in 1 ml) and 60 mls of ether and set aside 24 hours. Collect the precipitate in a Gooch crucible, wash with alcohol-ether (1 : 3) dry at 110°C. and weigh as pilocarpine picrolonate, of which 1 gm. corresponds to 0.44067 gm. pilocarpine.

412. **The galenical preparations** of pilocarpus are troublesome about formation of emulsions in shaking out with immiscible solvents. The U. S. P. prescribes for the fluid extract assay by the sawdust process (112), and this yields good results. See also (113). Chloroform alone is used for the immiscible solvent, otherwise the assay is made as in the case of fluidextract of belladonna leaves. The determination of alkaloid may be made by alkalimetric titration, or the alkaloid may be precipitated as a picrolonate exactly as in (411).

## POMEGRANATE BARK

413. **The bark contains** the alkaloids punicine, isopunicine, pseudopunicine and methyl-punicine, more or less volatile and unstable. A combination of these alkaloids with tannic acid is official (U. S. P. IX) under the name pelletierine tannate, pelleteirine being in fact a synonym for punicine. Valuation of the bark is based on its content of total alkaloid, the standard adopted by the pharmacopoeias varying from 0.25 percent (Codex) to 0.4 percent (German Pharmacopoeia) and 0.5 percent (Swiss Pharmacopoeia).

414. **Assay process of E. Schmidt\***. Place in a flask 12 gm. of the dried (over quick lime) sample in moderately fine powder, add 120 gm. of a mixture of ether and chloroform (3:1), shake well and add 10 mls of 10 percent solution of sodium hydroxide. Let stand 3 hours with frequent vigorous shaking,

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\*Lehrbuch Pharm. Chemie (Organische) II, 1584.

add 10 mils (or q. s.) of water, and shake until the powder aggregates. After standing 1 hour, decant 100 gm. of the clear ether-chloroform, evaporate in an air current to about 20 mils, transfer to a separator, rinsing the container 3 times with ether (5 mils) which is added to the separator. Add 50 mils of one hundredth normal hydrochloric acid, shake together, filter the acid aqueous solution through a small moistened filter, shake out the ether-chloroform twice with water (10 mils) which is passed through the same filter, titrate the excess of acid with hundredth-normal sodium hydroxide solution in presence of ether, iodeosin being used as indicator. Each mil of hundredth-normal acid corresponds with 0.001475 gm. of the mixed alkaloids of pomegranate bark contained in 10 gm. of the sample.

415. **Method of Erich Ewers\*** (modified). Put into a 200 mil flask 6 gm. of the powdered bark with 120 mils of a mixture in equal volumes of ether and petroleum benzin, add 10 mils of 20 percent solution of sodium hydroxide and shake vigorously at frequent intervals during one hour. Add 20 mils of water, shake and let stand 15 minutes. Decant through absorbent cotton 100 mils of the ether-benzin solution, transfer to a separator, add a few drops of methyl red indicator followed by decinormal sulphuric acid sufficient to supersaturate as shown by the indicator. Add then 10 mils of a 5 percent solution of sodium bicarbonate and shake well one minute. When complete separation of the fluids has taken place, draw off the alkaline solution into a second separator and shake with 20 mils of ether-benzin, which is to be separated and added to the alkaloidal solution in the first separator. Wash this with 10 mils of water; when separated draw this off and decant 96 mils of the ether-benzin; filter this through a dry filter into a 150 mil flask, washing the filter with ether-benzin, add five mils of tenth-normal sulphuric acid and shake well. Draw off the aqueous solution, wash the ether-benzin with two successive portions (10 mils) of distilled water, finally titrate excess of

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\*Arch. Pharm., 1899, 237, 49-57.



acid in the combined aqueous solution and washings with lime water (70), ascertain thus how much of the decinormal acid has been consumed in neutralizing the alkaloids. Each mil of decinormal acid is taken to correspond to 0.0141 gm. of the alkaloids pelletierine and isopelletierine present in 4 gm. of the sample.

416. If it is desired **to determine total alkaloids** in the bark, the assay process may be abbreviated by making the determination at once in the first ether-benzin solution, using 100 mils of this and omitting succeeding details up to the words "filter this" in line 20 of (415). The result will be the total alkaloids estimated as pelletierine, contained in 5 gm. of the sample.

417. **Assay method of Swiss Pharmacopoeia.** The same general plan is followed as in the foregoing, but ether alone is used as the immiscible solvent. An aliquot of the ether solution representing 5 gm. of pomegranate bark is reduced to about 15 gm. by distillation, to the residue is added 10 mils of water and 5 mils of absolute alcohol with 3 drops of haematoxylin indicator and the solution is titrated with tenth-normal hydrochloric acid. When the color changes to red brown, 30 mils of water are added, and the titration is continued, with vigorous shaking after each addition of the acid, until the color becomes citron yellow. Since the alkaloids present are volatile, the ordinary procedure of evaporating the alkaloidal solution to dryness of course cannot be followed in this or the foregoing assays.

418. **Assay of Pelletierine Tannate** (U. S. P. IX standard). The salt (0.5 gm.) is dissolved in potassium hydroxide T. S. (5 mils) and the alkaloid shaken out with chloroform (10, 5, 5, 5 mils). The combined chloroform solutions are slightly supersaturated with hydrochloric acid and evaporated to apparent dryness; the residue is dissolved in 5 mils of alcohol, the solvent evaporated and the alkaloidal hydrochlorides dried one hour at 60° C. and weighed. The weight is at least 20 percent of that of the sample assayed.

**SANGUINARIA**

419. **Bloodroot contains**, according to Schmidt and his associates, five distinct alkaloids, viz. sanguinarine (producing red salts), chelerythrine (yellow salts), protopine and beta- and gamma-homochelidonine (colorless salts). The medicinal activity of these several alkaloids has not been thoroughly investigated. An arbitrary standard for the drug has been assumed, based on the proportion of total benzin-soluble alkaloid. In the assay of the drug it is to be borne in mind that hydrochloric acid fails to extract the alkaloids completely from a chloroformic solution—that on the contrary chloroform removes from a solution containing hydrochloric acid in excess a portion of the alkaloids. Sulphuric acid may be used, but an organic acid (e. g., acetic or citric) is to be preferred.

420. **A practical assay method** which gives consistent results is that employed by V. O. Homerberg and G. M. Beringer.\* Mix 2 gm. of air-slaked lime with 7 mls of water to form a homogeneous magma. Incorporate with this 2 gm. of sanguinaria in fine powder. Evaporate to dryness on a water bath, powder, and transfer to a small percolator the orifice of which has been closed with a pledget of paper pulp moistened with a mixture in equal volumes of alcohol and ether. Percolate slowly with the same menstruum until a drop of the percolate evaporated on a watch crystal with a drop of tenth-normal hydrochloric acid gives no precipitate with Mayer's reagent. Shake out the percolate with three portions of a twenty percent solution of citric acid (25, 15 and 10 mls) and continue the extraction with 5 ml portions of the acid solution until Mayer's reagent gives a negative result with one drop of the latter. Shake out the combined acid solutions with petroleum benzin†, after rendering alkaline with a solution of sodium hydroxide, evaporate the solvent on a water-bath,

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\*Am. Journ. Pharm., 1913, 395.

†See footnote to (47).

dry to constant weight and weigh. The drug should yield 3 to 4 percent of benzin-soluble alkaloid but no standard is provided by the U. S. P. IX. For the assay of a tincture or fluidextract, evaporate to dryness a quantity of the preparation corresponding with 2 gm. of drug, mix with lime magma and proceed as above.

## THEOBROMINE

421. According to W. O. Emery and G. C. Spencer\* Theobromine, whether by itself or in admixture with alkaline salicylates, benzoates or acetates, may be determined with satisfactory precision by the periodide method (87). Under specified conditions one of which is the presence of some sodium chloride, a solution of theobromine in glacial acetic acid yields with a fixed volume of Wagner's reagent on addition of a mineral acid a crystalline periodide having a constant composition of  $C_7 H_8 O_2 N_4 H I_4$ . Titration of the excess of iodine makes determination of the alkaloid a matter of simple calculation (factor 0.0045025).

422. The method is as follows: Dissolve in a 30 mil Erlenmeyer flask approximately 0.1 gm. of the sample, accurately weighed, with its molecular equivalent of sodium acetate, (unless already in combination with an alkaline benzoate or salicylate) in 2 mls of glacial acetic acid by aid of a gentle heat. Add 3 to 5 mls of hot water. Transfer the solution, which must be perfectly clear, to a 100 mil glass stoppered measuring flask, by aid of several portions of warm water. Add at once 50 mls of tenth-normal iodine solution, followed by 20 mls of a saturated solution of sodium chloride. Finally add 2 mls of concentrated hydrochloric acid while rotating the flask, stopper, invert the flask 2 or 3 times and let stand 12 hours. Make up to the mark with water and mix thoroughly. Pass the liquid through a 5.5 cm. filter previously fitted to the funnel by wetting and then drying, reject the first 15 mls, then collect 50 mls

\*Journ. Ind. & Eng. Chem., 1918, 605, 6.

for titration with tenth-normal sodium thiosulphate. Subtract the number of mls used from 50 and multiply the remainder by 0.009005 to find the quantity of theobromine present in the sample taken. (The authors promise to report soon on a similar assay process for theophylline.)

**423. Method of E. Anneler\*** (for theobromine sodio-salicylate). Dissolve 1 gm. of the sample in 10 mls of water and rinse into a separator with a little more water, add 3 mls of 10 percent hydrochloric acid and a drop of phenolphthalein indicator, followed by a quantity of concentrated solution of barium hydroxide just sufficient to produce a permanent red color. Shake out with 20, 10, 10 and 10 mls of a solution of 1 part of phenol in 4 parts of chloroform. Evaporate off the solvent on the water bath, dry and weigh as pure theobromine.

**424. Method of Siegfried†.** The sample is dissolved as in Anneler's method, made acid with hydrochloric acid and shaken out in a separator with ether, which extracts salicylic acid. This may be determined by weight or titration. The theobromine remains suspended in the aqueous layer, and can be collected on a tared filter, dried and weighed. A correction should be made for solubility, so that the method is not to be approved. This is substantially the assay official in B. P. 1914, which makes a requirement of not less than 40 percent of theobromine (no correction for solubility) and not less than 35 percent of salicylic acid.

**425 For other methods** of determining theobromine, see chapter on Cacao (217) and (218). The U. S. P. IX gives the following method of determining the alkaloid in theobromine sodio-salicylate, similar to that of the German Pharmacoeia. To 2 gm. of the dried salt, dissolved in 10 mls of warm water, normal hydrochloric acid is added until the solution is neutral (phenolphthalein as indicator). Not more than 5.5 mls of the acid should be required. A drop

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\*Pharm. Ztg., 1910, 205.

†Jaggi in Schweiz. Wochenschr. f. Chem. u. Pharm., 1910, 569.

of 2 percent solution of ammonia is added and the mixture is set by for 3 hours at a temperature of 20° to 25° C. with occasional stirring. The precipitate of theobromine is collected on a tared filter, washed with 4 successive portions of ice water (5 mls) dried and weighed. A correction of 0.13 gm. for solubility is added. The corrected result should be not less than 46.5 percent of the weight of the sample taken. The German Pharmacopoeia makes no correction for solubility and requires a minimum of only 40 percent, although the salt is said to contain "about 45 percent" of theobromine.

## TOBACCO

426. **Nicotine, the principal alkaloid** of tobacco is so volatile that it can be distilled in a current of steam. Determination of the alkaloid has been commonly made by distillation, the method of Kissling being that most commonly followed and that adopted by the A. O. A. C. The procedure is as follows: Mix 20 gm. of finely powdered tobacco with 10 mls of a solution of sodium hydroxide 6 gm. in water 43 mls and alcohol 57 mls. Exhaust the mixture with ether 5 hours in a soxhlet apparatus. Evaporate the ether by a gentle heat, ammonia being thus practically eliminated. Take up the residue with 50 mls of an aqueous solution of sodium hydroxide 4:1000, transfer by aid of water to a 500 ml distilling flask and distil in a current of steam, using a condenser through which water is flowing rapidly. Use a three-bend outflow tube, a few pieces of pumice and a small piece of paraffin to prevent bumping and frothing. Distil until all nicotine has come over, leaving in the distilling flask not more than 15 mls of liquid. Titrate the distillate with volumetric sulphuric acid using phenacetolin, cochineal or methyl orange as indicator. Each ml of tenth-normal sulphuric acid corresponds to 0.016213 gm. of nicotine. (In assay of tobacco extracts use a convenient quantity of the extract, which is to be mixed as above with 10 mls of alcohol-soda solution and then calcium carbonate is to

be incorporated to form a mass suitable for extraction in a soxhlet. Pyridine is often present in such extracts, and complicates the assay. See (429).

**427. Assay by silicotungstic acid**, originally suggested by G. Bertrand and M. Javillier\*, and more recently studied by Robert M. Chapin†. Azor Thurston‡ directs: Weigh out 5 gm. of the sample, transfer with aid of 25 mls of water to a 500 mil Kjeldahl flask, add 1 to 1.5 gm. of solid paraffin, a few small pieces of pumice and 5 mls of a 50 percent solution of sodium hydroxide, distil in a rapid current of steam through a condenser and adapter into 10 mls of dilute hydrochloric acid (1 : 3) contained in a 1500 mil flask. When distillation is well started, apply heat to the distilling flask, to keep the volume of the contained fluid as low as practicable. Distil over about 950 mls (until 2 mls of the distillate show no opalescence on adding a drop of dilute hydrochloric acid and a drop of silicotungstic acid). Make up to 1 liter (or a larger volume if necessary). Filter and transfer 100 mls (or a corresponding aliquot) to a 250 mil beaker, add 3 mls of dilute hydrochloric acid (1:3) or more if necessary to make the solution distinctly acid. Add 10 mls of a 12 percent solution of silicotungstic acid (or 1 mil for each 0.01 gm. of nicotine supposed to be present), stir thoroughly and let stand 18 hours. Collect the precipitate on an ashless filter and wash with dilute hydrochloric acid (1 mil concentrated acid to 1 liter of water) until the filtrate is free from silicotungstic acid. Ignite (moist) precipitate and filter in a platinum crucible, applying full heat of a Bunsen burner 15 minutes. Cool in a desiccator and weigh. Multiply the weight of the ash by 0.114 to find the quantity of nicotine contained in the aliquot taken.

**428. Determination of nicotine by precipitation as a periodide**, has been proposed, but the precipi-

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\*Bull. Soc. Chem., 1909, 241-8.

†U. S. Dept. Agri. Bureau Animal Industry Bulletin, 133.

‡Journ. Am. Pharm. Assoc., 1915, 438.

tate is not constant in composition\*, so that quantitative results are far from exact. E. P. Harrison and P. A. W. Self† make use of the reaction for the separation of nicotine from ammonia but this is accomplished equally well by precipitation with silicotungstic acid.

429. Determination of nicotine by the **Polariscope**. When pyridine accompanies nicotine, it is convenient to estimate the latter by polarimetry, as proposed in 1889 by Max Popovici‡. J. A. Emery§ proceeds substantially as described in (425) taking a polariscopic reading of the distillate on the sugar scale, and ascertaining the value of  $1^\circ$  on that scale experimentally with a solution of pure nicotine. According to his observation each degree of rotation, using a 40 cm. tube, corresponds to 0.12 percent of nicotine. If pyridine as well as nicotine is to be determined, the sample is treated as in Kissling's assay process. The distillate is titrated with volumetric acid, to ascertain total alkalinity. It is also polarized to determine the amount of nicotine. The alkalinity due to nicotine is deducted from the total alkalinity, and the remainder is taken to be due to pyridine. Each mil of tenth-normal acid corresponds to 0.007905 gm. of pyridine.

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\*Lancet, April 6, 1912.

†Pharm. Journ. and Pharmacist, 1912, 718-9.

‡Zeitsch. f. Physiol. Chem., XIII 5; Arch. d. Pharm., 1889 558-9.

§Journ. Am. Chem. Soc., 1904, 1113-9.

## VERATRUM

430. **Active principles of the drug.** The several species of veratrum contain a number of alkaloids which are as yet imperfectly known. In *Veratrum album*, Wright and Luff\* in 1879 found, besides jervine, two crystallizable alkaloids, which they named rubijervine and pseudojervine. In 1891 Salzberger† reported that he had discovered two more crystallizable alkaloids, which he named protoveratrine and protoveratridine, the former of which he found to be an exceedingly active poison. It is to be noted that the official "veratrine," a mixture of alkaloids obtained from *cevadilla*, is not the active principle of veratrum, although the alkaloid veratrine, one of the constituents of the official "veratrine" is perhaps found, together with the amorphous veratroidine, in the species of *Veratrum*.

431. ***Veratrum viride***, the species now official in the U. S. P., is very closely related botanically to *V. album*, and contains the same alkaloids although in different proportions. In the present state of our knowledge we cannot determine by a chemical assay the therapeutic value of a sample of the drug, which indeed is now used only to a very limited extent. No standard is fixed for *Veratrum viride* of the U. S. P. IX, neither was there a standard in U. S. P. VIII, when the official *Veratrum* included *V. album* as well as *V. viride*. Standards have been adopted by some manufacturers, based on the total content of alkaloid, but such standards are in the nature of the case wholly fallacious.

432. **Determination of total alkaloids.** The most important fact to bear in mind in assaying veratrum is that jervine forms with mineral acids very sparingly soluble salts. Hence acetic acid is to be chosen rather than sulphuric or even hydrochloric

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\*Pharm. Journ. and Trans., May 31, 1897.

†Arch. de Pharm., 228, 462; Med. Chron., April 1891.



acid in extracting the alkaloids from their solution in an immiscible solvent. The following is the procedure of G. Bredemann\*. Put into a flask 12 gm. of powdered drug with 120 mls of a mixture of 3 volumes of chloroform and 2 of ether, macerate 10 minutes and add 10 mls of a ten percent solution of sodium hydroxide. Shake frequently during 3 hours, add water sufficient to agglomerate the drug. Decant the chloroform-ether, shake with magnesium oxide and a few drops of water to obtain a clear solution. Extract 100 mls of the solution, equivalent to 10 gm. of drug, by shaking out with water containing acetic acid. From the acid solution, the alkaloids are extracted by making alkaline with sodium hydroxide and shaking out with chloroform-ether (3 : 2) and are finally dried at 100° C. and weighed.

433. **Determination of jervine**, although giving little information with regard to the activity of the drug, may be made by the following method, taking advantage of the insolubility of jervine nitrate in a solution of potassium nitrate. Dissolve the crude alkaloid obtained from the drug or from one of its galenical preparations, in dilute acetic acid, filter and add to the solution an equal volume of a saturated solution of potassium nitrate. Set by in a cool place 12 hours, collect the jervine nitrate on a pair of mutually counterpoised filters, wash with a solution of potassium nitrate and finally with a little water, drain, press between blotting paper, dry at 80° C. and weigh. Assuming that the salt is anhydrous, it contains 86.4% of anhydrous jervine. (The alkaloid may also be determined by converting it into the very sparingly soluble sulphate.)

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\*Apoth. Ztg., 1906, 31, 41-53.

## Chapter III

### Potent Drugs, Non-Alkaloidal

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#### CANTHARIDES

433 a. **The active constituent** of cantharides is cantharidin ( $C_{10} H_{12} O_4$ ), a readily crystallizable compound having the properties of an anhydride, capable of combining with alkalies to form soluble cantharidates. Its most active solvents are acetone (1 : 38), chloroform (1 : 65) and acetic ether (1 : 100). In cold water it is practically insoluble (1 : 30,000). Glacial acetic acid dissolves it rather freely (1 : 120) and it is soluble in fixed and volatile oils. Alcohol dissolves it so sparingly that tinctures (1:10) prepared even with dehydrated alcohol contain only a part of the active constituents of the drug. In determining catharidin advantage is taken of its disposition to separate from its chloroformic solutions, when evaporated in distinct crystals, practically insoluble in cold petroleum benzine.

433 b. **One of the best assay processes** is that devised by Eldred and Bartholomew.\* Place in cylindrical percolator, capacity about 100 mls, provided with a ground glass stopper and a glass stop-cock, 10 gm. of cantharides in No. 60 powder, add a mixture of 25 mls of chloroform and 2 mls of glacial acetic acid, stopper and macerate one hour. Then open the stop-cock and allow the solvent to drain away through a layer of absorbent cotton which has been provided, pack the drug firmly and exhaust by slow percolation with chloroform. Distil off most of the chloroform and blow air through the flask to remove the remainder. Add 10 mls of liquid petrolatum and 150 mls of hot water to the residue in the flask, and heat on a water bath nearly to the boiling

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\*Proc. Am. Pharm. Assoc., 1907, 360-4.

point, shaking the mixture repeatedly. Separate the aqueous solution, and treat the oily stratum as before with three successive portions of water, 75, 50 and 25 mils respectively.

433 c. Filter the aqueous solutions as they are drawn off into a separator, add a little hydrochloric acid, cool and shake out with four portions of chloroform. Evaporate the solvent by a gentle heat to a small volume, driving off the remainder by a current of air, taking care that there is no loss from decrepitation. Finally purify the crystals of cantharidin by washing with three portions (2.5, 2.5 and 1 mil), of a mixture of equal volumes of dehydrated alcohol and petroleum ether, saturated with cantharidin, pouring the washings through a small filter. Wash the crystals and filter with petroleum ether until the filtrate leaves no appreciable residue on evaporation. Wash the filter with a little chloroform, receiving the filtrate in the vessel containing the crystals of cantharidin. Evaporate the chloroform by a current of air and dry to constant weight at ordinary room temperature in a vacuum desiccator. The authors show, 1st, that in the foregoing assay process there is no appreciable loss of cantharidin in the evaporation of the chloroform used as a solvent, and 2nd, that there is material loss of cantharidin if the crystals are dried in open air at 65° or even at 40° C.

433 d. **Method of H. Emde\***, based on that of A. Kneip†. Cantharides, 15 gm., is mixed with 3 mils of alcoholic hydrochloric acid, 25 percent (made by passing gaseous hydrochloric acid into dehydrated alcohol). After 15 minutes contact at a temperature not above 50 C., the mass is extracted in a soxhlet with a mixture (30 : 50 vol.) of petroleum ether (boiling point 50°-75° C.) with benzene. The tared receiver also contains 50 mils of the same mixture. When extraction is complete, the solvent is distilled off and the last traces dissipated by a current of air. The residue is treated with 5 mils of a mixture (1:2 vol.)

\*Archiv. d. Pharm., 1911, 249-59; Jn. Pharm. Chim., 1911, 268.

†Archiv. d. Pharm., 1911, 259-85.

of dehydrated alcohol and petroleum ether to dissolve the green fat. After standing 30 minutes in a cool place, the liquid is decanted on to a dried tared filter. The crystals in the flask are washed with the above mixture (5, 5, 2.5 and 2.5 mls), these washings being run through the same filter, which is finally washed with 15 mls of the same mixture. The flask and filter are then dried at a temperature not exceeding 60° C. and weighed. (As shown by Eldred and Bartholomew, this involves some loss of cantharidin.) To determine the amount of **free cantharidin**, the same process may be carried through with omission only of the initial treatment with alcoholic hydrochloric acid. This datum however cannot be regarded as important.

433 e. **Method of U. S. P. IX.** In its essential features this agrees with the foregoing, but the manipulation is simplified to adapt it to the equipment of the pharmacists' laboratory. The cantharides (15 gm. in No. 40 powder) is placed in a strong bottle (capacity not less than  $\frac{1}{4}$  liter), 150 mls of a mixture of benzene, and petroleum ether (2:1 vol.) is added, followed by 2 mls of strong hydrochloric acid. After a maceration of 10 hours, the bottle, securely corked, is warmed gradually to 40° C. and maintained at that temperature, with frequent shaking, three hours. Of the cooled and filtered solution, 100 mls are evaporated rapidly in a tared beaker or flask to 5 mls, 5 mls of chloroform are added and the mixture is set by in a moderately warm place. When the solvent has evaporated the crystals are washed repeatedly with a mixture (1:1 vol.) of dehydrated alcohol and petroleum ether, saturated with pure cantharidin, until all fat and coloring matters have been removed, the washings being passed through a pellet of absorbent cotton. The final steps are identical with those in the method of Emde.

433 f. **Method of Self and Greenish\*.** In outline this consists in extracting 20 gm. of the drug, moistened with 3 mls of hydrochloric acid, in a

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\*Pharm. Journ., March 16, 1907, 324-8.

soxhlet with benzene, distilling off the solvent, recovering from the distillate any traces of cantharidin by shaking out with a 1 percent solution of potassium hydroxide, adding this, after acidifying, to the residue of cantharidin and fat in the distilling flask. The mixture is boiled with several successive portions of water, which extracts the cantharidin; hydrochloric acid (5 mils) is added to the united aqueous solutions, from which the cantharidin is removed by shaking out with chloroform. The further steps in the assay are those of the U. S. P. IX assay.

433 g. **Method of Puran Singhe.\*** One hundred gm. of cantharides are made into a pasty mass with 2 gm. of sodium hydroxide and a little water, dried and extracted with a dilute aqueous solution of alum. On evaporation, the cantharidin crystallizes from the concentrated solution together with basic aluminum salt, which is removed by hydrochloric acid. The cantharidin may be recrystallized from chloroform, dried with the usual precautions and weighed.

433 h. **Cerate of cantharides**, according to Eldred and Bartholomew† may be assayed by extracting 25 gm. in a soxhlet with a mixture of 150 mils of chloroform and 2 mils of glacial acetic acid, distilling off the chloroform and proceeding as in assaying the crude drug, omitting the liquid petrolatum.

433 i. **Tincture of cantharides**, according to the same authorities, can be assayed by adding to 100 mils of the tincture 2 mils of glacial acetic acid, distilling off the alcohol and completing the assay as in the case of the crude drug. There is no appreciable loss of cantharidin in the distillation.

433 j. **The following method** for assay of the tincture is of interest as following in the main the lines of the assay process for cantharides of the German Pharmacopeia (V)‡. To 50 mils of the tincture, add 25 mils of water and 0.5 gm. of sodium carbonate and evaporate to dryness on a water bath. Dissolve

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\*Imp. Forest Research Inst., Reprint 1907.

†Proc. Am. Pharm. Assoc., 1907, 360-4.

‡Dr. R. Gaze, Apoth. Ztg., 1911, 332-3.

the residue in 10 mls of water, add 2 mls of hydrochloric acid (25 percent), transfer to a separator and shake out with chloroform (10, 5, 5, and 5 mls). Evaporate the chloroform on a water bath and finally with aid of a bellows. At the end of 12 hours, wash the dry residue with petroleum benzin (10, 5, 5, 5 and 5 mls, passing the successive portions through a small filter, which is finally washed once with petroleum benzin. Allow residue and filter to become air-dry, then wash first with 10 mls of water containing one drop of solution of ammonium carbonate, followed by pure water, and dry at 50° C. (The German Pharmacopoeia continues the washing with water containing ammonia until the filtrate passes colorless, then follows with 5 mls of water). Dissolve the residue of crude cantharidin in a little acetone, pass the solution through the filter into a tared weighing flask and wash the former container and filter with several fresh portions of acetone. Evaporate the acetone, finally with aid of bellows, dry the residue at 50° C. to constant weight and weigh. (The German Pharmacopoeia dries at 100° C. In fact, heat above 35° C. should not be employed, the drying being best conducted in a vacuum desiccator.)

## CONVALLARIA

434. **Lily of the Valley**, which, like digitalis, is now classed as a cardiac tonic, contains two glucosides, convallarin and convallamarin, the latter of which appears to be the principle to which the characteristic medicinal activity of the drug is due. The former is scarcely soluble in water, yet sufficiently so to impart to it its acrid taste and the property of frothing when shaken. It dissolves freely in alcohol. Convallamarin is soluble in water and alcohol, but not in ether. It is characterized by a strongly bitter taste, afterwards sweetish, and has been assigned the formula  $C_{23}H_{44}O_{12}$ .

435. **The valuation of the drug chemically** must involve a determination of convallamarin, which may be attempted by Tanret's process for the isolation

of the glucoside, as follows\*. The drug is exhausted with alcohol, the tincture treated with lead subacetate and filtered; the excess of lead is removed by sulphuric acid, of which no more should be used than is necessary. Filter, neutralize carefully, evaporate off the alcohol. The residue is to be taken up with water, filtered and precipitated with a solution of tannin, care being taken to keep the solution neutral with sodium carbonate. The precipitate is washed carefully, dissolved in 60 percent alcohol, decolorized with animal charcoal, decomposed with zinc oxide, filtered and evaporated to dryness. To free it from saline impurities, dissolve it in 90 percent alcohol, filter again and evaporate to dryness.

436. The yield being very small—only 0.2 percent—it would be necessary to use at least 25 gm. of drug for an assay and the process probably does not yield the whole of the glucoside, but no better chemical assay has been suggested. The drug has therapeutic properties similar to those of digitalis, and is to be assayed biologically by the same methods employed for that drug, although it is to be understood that the assay is of value only for comparing one sample of convallaria with another, not for determining the dose equivalent to that of a standard sample of digitalis or of strophanthus. See comments under *Strophanthus* (449 and 450). According to Houghton, the toxicity of convallaria root is six times that of digitalis; of the flowers, ten times.

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\*Journ. de Pharm., (6) III, 355; Pharm. Journ., 1882, 423.

## DIGITALIS

**437. There has been much discussion** on the relative therapeutic value of the several glucosidal principles contained in digitalis. It is maintained, and may be conceded, that of these the most active (toxic) is digitoxin, but it is questionable whether this produces the beneficial effects for which the drug is prescribed, and which are best obtained from the infusion. Keller's assay process aims to determine digitoxin, assuming that this is the most important active principle in the drug. There is reason to believe, first, that this assumption is unwarranted by fact, and second, that the assay fails to accomplish its aim. However, it is still employed by some, although it has not been given a place in any of the leading pharmacopoeias. The activity (toxicity) of the drug can be determined with a good degree of precision by a biological assay, the "one hour frog" method described in the U. S. P. IX being that most often employed. See comments under (449) and (450) in the chapter on *Strophanthus*.

**438. Assay method of Keller\*.** Exhaust 15 gm. of the powdered leaves with 70 per cent alcohol by percolation. Evaporate, dissolve the residue in 140 mils water, and add solution of lead subacetate in slight excess. Remove the excess of lead from the filtrate with sodium sulphate, filter, measure 100 mils of the filtrate, make alkaline with ammonia and shake out with several portions of chloroform. Evaporate, dry the residue and weigh as crude digitoxin. Dissolve this in 3 gm. of chloroform, mixed with 7 gm. of ether and pour the solution into 50 gm. of petroleum benzin. The digitoxin segregates in flakes which are caused to separate by vigorous stirring of the mixture and are then collected, dried and weighed as "pure" digitoxin.

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\*Chem. Centr., 1897, I, 1211; from Ber. pharm. Ges. 1895, Heft 11.



439. The method of J. Burmann\* extracts a mixture of glucosides (pseudo-digitoxin and digitoxin) which may probably be taken as a measure of the toxicity of the drug, but more convincing evidence than we yet have that therapeutic activity is in proportion to toxicity is necessary before it can be made the basis for standardization of so potent a medicinal agent. An aqueous solution is first prepared, which is submitted to dialysis, treated with solution of lead sub-acetate after addition of alcohol and the excess of lead removed with sodium phosphate. The solution is concentrated, extracted with chloroform and the chloroformic solution mixed with ether and then with petroleum benzin, which precipitates the glucosides. The original paper should be consulted for details.

440. **Empirical test of W. H. Martindale†.** This is a colorimetric adaptation of Fröhde's test. Prepare a tincture of the drug, (1:10), with 70 percent alcohol. Mix 10 mls of this with 10 mls of water, add 3 mls of a 10 percent solution of lead acetate, filter, remove excess of lead with dry sodium phosphate (0.2 gm.), filter once more, add 0.2 gm. of calcium carbonate and evaporate to dryness. Extract the residue with chloroform five times, evaporate off the solvent and extract the residue with warm water, evaporate and extract the residue with cold chloroform, evaporate and dissolve the residue in 4 mls of glacial acetic acid. Mix 0.1 ml of this solution with 1 ml of Fröhde's reagent (strong sulphuric acid containing in each ml 0.01 gm. of ammonium molybdate), let stand five minutes, then compare the color of the solution with that produced by drug of standard strength which has been treated in the same manner. It is assumed that the depth of tint is proportioned to the amount of water-soluble glucosides present.

441. **Standardization of galenical preparations futile.** Digitalis is a drug subject to deterioration, and the same is true of the extract, the infusion and of tinctures, particularly those made with strong

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\*Bull. Soc. Chim., 1912, 221.

†Pharm. Journ., 89, 738, 745-8, 778-80

alcohol. Until some way has been found to prevent such deterioration, any standards provided for such preparations, or even for the drug itself, are worthless for securing permanent uniformity of dosage. It is maintained by some that a fat-free tincture is reasonably permanent, but experimental data are as yet insufficient to establish the fact.

## SANTONICA

442. **The active constituent, santonin** ( $C_{15}H_{18}O_3$ )\* may be determined by the following method adapted from that of Fromm<sup>†</sup>. Macerate 15 gm. of santonica in moderately fine powder with 100 mls of chloroform in a separator 12 hours, shaking occasionally. Draw off through a pledget of absorbent cotton 68.5 mls of the chloroform, transfer to a 200 mill Erlenmeyer flask and evaporate to about 7.5 gm. Add 100 mls of a five percent solution of barium hydroxide, place in a hot water bath until resin rises to the surface, and continue to apply heat until all chloroform is driven off. Filter through a well wetted filter, rinse the flask and filter twice with hot water (10 mls), add to the filtrate 4 mls of concentrated hydrochloric acid and heat 15 minutes on a boiling water bath. When cool transfer to a separator, rinse the container with 20 mls of chloroform which is to be added to the separator, shake two minutes and let separate. Draw off the chloroform and filter through a dry filter into a beaker. Shake out the aqueous solution in the separator with two additional portions of chloroform (15 mls) which are to be drawn off and filtered as before, evaporate the chloroform by aid at the end of a current of warm air, dissolve the residue in 10 mls of alcohol and add 42 mls of hot water. Filter the milky liquid at once into a tared 100 ml Erlenmeyer flask and wash beaker and filter twice with 10 mls of a mixture of 4 mls of absolute

\*The anhydride of santoninic acid; a crystallizable compound very readily soluble in chloroform, combining with alkalies to form soluble santoninates.

†Caesar and Lortetz Bericht, Sept., 1912. See also Drugg. Circ. 1913, 57, 448 and Journ. Am. Pharm. Assoc. 1914, 3, 635.

alcohol and 18 mls of water. Rub the walls of the flask with a glass rod to start crystallization, then set the flask aside for 24 hours. Collect the crystals of santonin on a tared filter, wash twice, using each time 10 mls of the above mixture of alcohol and water, dry filter and flask with contents to constant weight at 100° C., add 0.04 gm. as correction for solubility, to find the weight of santonin contained in 10 gm. of drug.

## SQUILL

443. As in the case of *digitalis*, there remains much to learn regarding the active constituents of squill. The drug acts on the heart in much the same way as *digitalis*, and the same methods of biological assay are employed in estimating the relative strength (toxicity) of different samples. By the "one hour frog" method of the U. S. P. IX, standard squill and *digitalis* are equally potent, *strophanthus* being normally one hundred times as toxic. For comments on this assay method see (449) and (450).

444. A **chemical assay** for total glucoside might be of value, but none seems to have been worked out. On general principles one might proceed substantially in the following manner: Macerate the drug 24 hours with water containing 2 percent of solution of lead subacetate. From an aliquot portion of the solution remove the excess of lead by addition of sodium phosphate or sodium sulphate, filter, evaporate an aliquot to a small volume and precipitate it with tannic acid. Dissolve the precipitate in alcohol, evaporate the solution with addition of zinc oxide to dryness. Exhaust the residue with boiling dehydrated alcohol (or perhaps with acetone) and evaporate the solution to dryness, to obtain the crude glucosides.

## STROPHANTHUS

445. **The active constituent** in the various species of strophanthus is glucosidal in nature, but not identical in the different species. Much remains to be learned regarding the several glucosides, which unquestionably differ greatly in therapeutic activity. Under the circumstances no chemical assay can be considered as a basis for a standard for strophanthus and its preparations. A biological assay alone can show the relative therapeutic activity of different samples. The seeds of but one species of strophanthus viz. *S. Kombe*, are official in the British, German and other leading pharmacopoeias, but the U. S. P. IX recognizes also *S. hispidus*, although the official strophanthin is said to consist of a glucoside or a mixture of glucosides obtained from *S. Kombe*. If the official drug consisted of the seeds of this species alone, there is reason to believe that a chemical assay by the method given in the following paragraph would give results pretty closely coinciding with those obtained by a biological assay.

446. **Assay method of Fromme (1910).** In a prize essay on the various methods proposed for the assay of strophanthus, J. B. Lampart and A. Mueller sum up their conclusions as follows:\* The assay methods suggested by Fraser, Fromme (1897), Fromme (1900), Krosberg, Thoms, Mann and Dohme for the drug, and those by Dowzard and Barclay for the tincture, are not satisfactory, while the assays of Modeen, Fromme (1905), Fromme (1910) and Haycock are practical enough to be usable. Of these four, that of Fromme (1910) is the best.

447. **The process is conducted as follows:** Heat one hour under a reflux condenser 7 gm. of the powdered sample with 70 gm. of dehydrated alcohol. When cool, add alcohol if necessary to restore to the original weight. Evaporate 50.5 gm. of the fluid, representing 5 gm. of drug, to dryness, treat the

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\*Arch. Pharm., 251, 609.

residue repeatedly with petroleum benzin to remove oil, take up the residue with 8 mls of boiling water, add 5 drops of solution of lead acetate and 0.2 gm. of infusorial earth, pour the mixture on a filter, wash the residue with boiling water until the filtrate is free from bitterness. Add to the solution 0.15 ml of hydrochloric acid, heat to boiling on an asbestos plate over a small flame and maintain a boiling temperature 2 hours, adding water if the volume becomes less than 10 mls. (The object of this prolonged heating with hydrochloric acid is to convert strophanthin into strophanthidin). Cool the solution, make up with distilled water to about 20 mls, shake out with two portions of chloroform (10 mls each), which are filtered into a tared Erlenmeyer flask. Heat the aqueous solution again half an hour, and when cool shake out with 3 additional portions (10 mls each) of chloroform, which also are filtered into the flask. Evaporate off the chloroform, dry the residue in a desiccator and weigh as strophanthidin, multiply the weight by 2.187 (sic)\*, to find the quantity of strophanthin in 5 gm. of the sample. By this method of assay strophanthus yields 4.5 to 7.0 percent of strophanthin.

448. **In case of a tincture** of strophanthus, evaporate 50 mls on a water bath nearly to dryness, add boiling water, solution of lead acetate and infusorial earth as above and go on with the assay precisely as there detailed.

449. **Biological Assay of Strophanthus.** The U. S. P. IX supplies an assay process for the heart tonics, convallaria, digitalis, strophanthus and squill, in which the lethal dose of each of these drugs is brought into comparison with that of ouabain, a crystallizable glucoside derived from *Arcocanthera Ouabaio*, a plant of the same family as strophanthus, the same glucoside existing also in *Strophanthus glaber*. While this glucoside is said to vary in activity it more nearly approaches uniformity than any other derivative yet known from any of the drugs of this

\*The correct factor (following E. Schmidt) would seem to be 1.773. Ed.

class. The principle of the assay is simple enough. By repeated trials the minimum quantity of the drug that will stop the heart's action in a frog of standard weight in one hour is ascertained. This quantity in case of a drug of standard strength is just twelve times that of the corresponding minimum dose of ouabain under the same conditions. It is not to be inferred that therapeutically one milligram of ouabain is equivalent to twelve milligrams of strophanthus, any more than it is that one mg. of strophanthus is the therapeutic equivalent of 100 mg. of digitalis or of squill. Yet different samples of any of these so-called heart tonics can be compared with others of the same drug through this test of toxicity.

450. **Authorities differ widely** with regard to the relative toxicity of the several heart tonics as determined by frog tests. The following table shows the minimum lethal doses (M. L. D.) given resp. by the U. S. P. IX (one-hour frog) method and by Dr. Houghton's\* (12-hour frog) method. According to the U. S. P. the relative toxicity of digitalis, squill and strophanthus is 1.00, 1.00 and 100.00; according to Houghton it is 1.00, 1.25 and 200.00, convallaria (U. S. P. VIII) being 6.00.

### Minimum Lethal Dose of Heart Tonics.

Drug or Preparation	U. S. P. IX	E. M. Houghton
Onuabain.....	0.0000005	
Kombe strophanthin.....		0.000001
Convallaria		
Rhizome and roots.....		0.00025
Herb.....		0.00015
Flowers.....		0.00009
Digitalis		
Fluidextract.....	0.0006	0.0015
Extract.....		0.0005
Tincture, U. S. P.....	0.006	0.015
Digitalin (German).....		0.00005
Squill		
Fluidextract.....	0.0006	0.0012
Strophanthus		
Seed.....	0.000006	0.0000075
Tincture, U. S. P.....	0.00006	0.00007

\*The Lancet, June 19, 1909.

## Chapter IV

### Cathartic Drugs

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#### ALOES

451. **The several varieties** of aloes contain crystalline principles called aloins, which are not identical in the several varieties, but are evidently closely related chemically. The aloins are certainly actively cathartic, but they are not the only cathartic principles present in the drug. The subject is as yet imperfectly understood, so that absolute standards cannot be established. The procedures which follow serve a more or less useful purpose in the approximate valuation of samples of the drug.

452. **Assay method of L. van Itallie\***. Determination of non-resinous matters, based on method of Tschirch and Hoffbauer. Five gm. of the powdered drug are heated in a 50 mil flask with 5 mls of methyl alcohol until a homogeneous liquid is produced. After cooling to 60° C., add gradually 30 mls of chloroform and shake the mixture vigorously five minutes. When quite clear pour off the chloroform into a distilling flask. Drive off residual chloroform from the residue and extract this again three times in succession with methyl alcohol and chloroform precisely as before. Distil off the solvent from the combined solutions, dry the residue to constant weight at 100° C. and weigh. The activity of the aloes is roughly in proportion to the percentage of non-resinous matters. The yield in the tests reported by Tschirch and Hoffbauer was resp. from Cape aloes (soft) 86.8%, from Cape aloes (dry) 81.2%, from Uganda aloes 80.4%, from Barbadoes aloes 72.4%, from Curaçao aloes 66.6%, from Socotrine aloes 36.6%. The results reported by van Itallie showed for Cape aloes 82.0 and 56.2%; for Curaçao aloes, 86.4, 88.6 and

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\*Pharm. Weekblad, 1905, 42, 553-60.

78.3%; for Aruba aloes, 61.0%, indicating great variability in the drug.

453. **E. Léger\*** separates the aloin from the above mixture of non-resinous substances by dissolving it in just sufficient methyl alcohol to form a syrupy solution and leaving this exposed to the air in a cool place several days, when the aloin crystallizes out. The proportion of aloin in the residue may be determined approximately by the colorimetric method of Schouteten. An aloin solution produces in a strong solution of sodium borate an intense green fluorescence which develops within a short time. In a solution containing one part in 250,000, a faint suggestion of green is distinguishable in looking down a comparison tube of white glass filled to a height of 12 cm. (say 5 inches). When the proportion is reduced to 1:300,000 this is no longer perceptible. In practice, standard solutions 1:250,000 and 1:200,000 should be prepared for comparison. Treat the residue with warm water, which will not dissolve it completely. Without filtering add 400 mls of a saturated aqueous solution of borax, dilute until a portion of the solution shows the same amount of fluorescence as the standard solution, and calculate from the volume of the diluted solution the quantity of aloin present.

454. **The residue contains besides aloin** a large proportion of anthraquinone derivatives, to which in part the medicinal activity of the drug is believed to be due. To determine these, the author proceeds as follows: Weigh 1 gm. of the aloes, treat this in a porcelain capsule on a water bath with 20 mls of concentrated nitric acid, covering the capsule at first, during the period of violent reaction, with an inverted funnel. After two hours the funnel may be removed, and the solution evaporated. Add to the residue 2 mls of concentrated nitric acid and heat two hours, adding occasionally more acid to replace that evaporated, finally bringing the residue to dryness. Treat this with water which produces a yellow solution, leaving the chrysamic acid which has

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\*Journ. Pharm. Chem., (6) 15, 519.



been formed undissolved. Collect this on a glass wool filter and wash it with a few drops of water. Add the water drop by drop until the color of the filtrate changes from yellow to red. Dissolve any of the chrysamic acid remaining in the capsule in water containing ammonia, and with this dissolve also the chrysamic acid on the filter, using more ammonia if necessary. Finally make up the volume of the solution to 1 liter, and determine approximately the quantity of chrysamic acid it contains by colorimetric comparison with a solution of pure chrysamic acid (2 mg. to the liter), containing some free ammonia.

## ELATERIUM

455. **The active principle** of the drug is **elaterin**,  $C_{20}H_{28}O_5$ , a readily crystallizable substance of a bitter disagreeable taste. It is almost insoluble in water or glycerin, and, according to the U. S. P. VIII, requires for solution at 25° C. 318 parts of ether, 262 parts of alcohol and 22 parts of chloroform. The drug, which is now seldom used, is a very crude product, very variable in strength, often containing a large proportion of mineral matter (calcium carbonate, terra alba, etc.) If of good quality, it yields on ignition not more than 9 percent of ash.

456. **Assay for elaterin.** Put into a small flask 2 gm. of the drug in fine powder with 15 mls of chloroform, and macerate half an hour at a temperature of about 55° C. Transfer to a small filter and when all the chloroform has passed, percolate with fresh chloroform to complete exhaustion. Evaporate the solution in a tared capsule, dry at 100° C. and weigh. Dissolve the residue in 15 mls of ether, added at once, transfer immediately to a small tared beaker, cover, and set by to crystallize. The ether forms momentarily a perfect solution of the residue, from which crystals of elaterin begin almost immediately to separate.

457. After a few hours decant the ether into a second tared beaker and evaporate to about 3 mls. Decant the remaining ether into a third beaker,

leaving the crystals of elaterin which have formed, wash these with a little ether by decantation, adding this to the third beaker. Allow the ether in the third beaker to evaporate entirely, add 3 mls of fresh ether and observe whether any crystalline residue remains undissolved. If so, this must be washed with a few drops of ether. Finally dry all the crystals obtained at 100° C. and weigh. The weight of the elaterin is usually about one half that of the original chloroform extract. If it fall much short of that, loss of elaterin in the crystallization may be suspected. In any case it is safe to add a solubility correction of 10 mg. Elaterium of good quality should yield 16 to 20 percent of crystallized elaterin.

## IPOMOEAE

458. **Mexican Scammony**, the dried root of *Ipomoea orizabensis* Lidanois, is now official in the United States and British Pharmacopoeias, in the latter under the title *Orizaba Jalap root (Ipomoeae radix)*. The resin from this drug, like that from true scammony, is soluble in ether. Assay of the drug can no doubt be made by the several methods employed for jalap. See (459) to (464). The drug yields a larger percent of resin than jalap, but no standard has as yet been established, and chemical data with regard to the therapeutic efficiency of the resin as compared with that of scammony are as yet meager.

## JALAP

459. **The active constituent of jalap is its resin.** The proportion of this varies greatly. Minimum limits are set by the leading pharmacopoeias ranging from 7 percent (U. S. P. IX) to 10 percent (Ph. G.) The following are among the most approved methods of assay.

460. **Preference is to be given**, on account of its simplicity and the uniformity in the results it gives in different hands, to the U. S. P. assay. Exhaust 10 gm. of jalap in No. 60 powder by percolation with

alcohol, collecting 100 mls of percolate. Measure into a separator 20 mls of the percolate, representing 2 gm. of drug, add 20 mls of chloroform, shake together, add 20 mls of distilled water and once more shake thoroughly. When the liquids have separated completely, draw off the lower stratum into a tared beaker, wash the residual fluid by rotating a moment or two (not shaking) with 5 mls additional of chloroform, which is drawn off and added to the beaker. Evaporate the solvent on a water bath, add about 2 mls of alcohol, evaporate once more, dry to constant weight at 100° C. and weigh.

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**460½. Potassium Citrate method of W. L. Scoville\*.** The foregoing method, according to Scoville, extracts less than 95 percent of the resin present in the drug. Practically the whole of the resin can be extracted by the following modified procedure. Percolate 10 gm. of the drug with U. S. P. alcohol to obtain 100 mls of tincture. Mix 20 mls of this tincture in a separator with 10 mls of chloroform, add 20 mls of a concentrated solution of potassium citrate (300 gm. of the citrate in 180 mls of water), shake well and set aside over night to secure complete separation. Draw off the lower layer and reject it. Decant the upper layer into a tared beaker. Rinse the separator with a mixture of chloroform 2 mls and alcohol 4 mls, and add this to the beaker. Evaporate, dry to constant weight at 100° C. and weigh as total resin from 2 gm. of drug. (In a fluid-extract, mix 2 mls of the sample with 18 mls of alcohol; 10 mls of chloroform and 20 mls of the citrate solution.) It is to be noted that Scoville found by precipitation with water, only about 84 percent of the quantity of resin found by the potassium citrate method, but details are not given of the precipitation method he employed.

**461. Method of G. Fromme†.** Boil 7 gm. of powdered jalap with 70 gm. of dehydrated alcohol under a reflux condenser two hours. When cool,

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\*Bull. of Pharm., May, 1909.

†Pharm. Ztg., 1905, 773, from Caesar and Loretz's Report.

restore the original weight of the mixture with dehydrated alcohol and filter off 51 gm. of the tincture into a tared porcelain dish. Add a few gm. of water, evaporate off the alcohol, add 50 mls of hot water and knead the separated resin with a glass rod so as to form a coherent mass. This operation is repeated twice with fresh portions of hot water, the resin is dried on a water bath at 100° C. and finally in the exsiccator to constant weight.

**462. Method of the German and Swiss pharmacopoeias.** Digest 10 gm. of finely powdered jalap with 100 mls of 90 percent alcohol 24 hours at about 30° C., with frequent shaking. From 50 mls of the clear solution, evaporate the alcohol, and wash the residue with warm water as long as this extracts color from it. Dry the resin on a steam bath and weigh.

**463. Assay of fluidextracts or tinctures.** The simplest procedure is to evaporate to dryness a quantity of the preparation representing about 5 gm. of drug, after adding half its volume of distilled water. When the alcohol has been nearly all driven off and the resin has adhered to the bottom and sides of the dish, add about 50 mls of hot water, and continue the heating if necessary until the water is quite clear. Decant the water, redissolve the resin in a few mls of alcohol, add 20 mls of hot water and evaporate once more until the resin has separated, leaving a clear solution. Decant, and wash the resin once or twice with hot water, dry at 100° C. and weigh.

**464. Otherwise** adapt either one of the foregoing methods, giving preference to that of (460) or (460½) if the preparation is made with strong alcohol. In the case of a fluidextract, add to 2 mls of the sample 18 mls of alcohol and 20 mls of chloroform, shake together, add 20 mls of distilled water, shake and let separate, completing the assay as in (460).

N. B. For Phenolphthalein, which may be classed as a cathartic agent, see (741) et seq.

## PODOPHYLLUM

465. **The official drug** is derived from the American plant, *Podophyllum peltatum* Linné. A species native in southern India, *P. Emodi* Wallich, has similar properties but is much more active. The resins precipitated from an alcoholic tincture of the former constitute the drug commonly known as podophyllin, the *Resina Podophylli* of the U. S. P. The active constituent of this "resin" is a crystallizable principle having the empirical formula  $C_{15}H_{14}O_6, 2H_2O$ . (Dunstan and Henry). It was first obtained in crude form by Podwysotski, by extracting the drug with chloroform and precipitating the concentrated solution with petroleum benzin. By the action of alkalis podophyllotoxin is converted into the isomeric picropodophyllin, a readily crystallizable substance having little if any purgative activity. It is optically inactive, while podophyllotoxin is strongly laevorotatory. The Indian drug is said to contain in addition to podophyllotoxin a strongly purgative resin (podophyllo-resin).

466. **Standardization of the American drug** might be rationally based on its content of podophyllotoxin. No practical method however has yet been devised for separating this constituent quantitatively from the drug. Possibly it might be extracted in crude form by the original method of Podwysotski and then determined by its optical rotation, but it would be necessary to make sure that the crude product contained nothing having optical activity except podophyllotoxin.

467. **Determination of Podophyllotoxin.** It is possible however to convert the podophyllotoxin into the isomeric picropodophyllin, which can be easily determined. Dunstan and Henry\* propose the following procedure: Mix a convenient quantity of the powdered drug, dried at  $100^{\circ}C.$ , with lime and extract in a soxhlet apparatus with chloroform,

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\*Journ. Chem. Soc., 1898, Trans., p. 224.

evaporate, dissolve the residue in absolute alcohol, add slaked lime to form a thin paste and evaporate to dryness. Add more alcohol and evaporate again, then boil the residue with absolute alcohol and filter hot. Introduce filter and contents into a soxhlet and extract with absolute alcohol. Evaporate the mixed alcoholic solutions to complete dryness and weigh as picropodophyllin (anhydrous). The product usually retains a small proportion of lime, which may be determined by ignition and deducted from the gross weight. The method may be employed for the Indian as well as the American drug, but it is to be remembered that the former contains another purgative constituent in addition to the podophyllotoxin.

**468. Assay of podophyllum for resin.** For practical purposes the medicinal value of podophyllum may be measured with sufficient exactness by determining its content of resinous constituents. This may be readily done by the method of W. M. Jenkins.\* Put into an Erlenmeyer flask fitted with a reflux condenser 10 gm. of the drug in No. 60 powder with 25 mls of alcohol and heat three hours on a water bath at 80° C. Transfer the contents of the flask to a small percolator, rinse the flask with alcohol, which is then used to percolate the marc. Percolate with alcohol to obtain in all 50 mls. Transfer an aliquot portion of this to a separator, add an equal volume of chloroform, also of water containing 0.6 percent of hydrochloric acid (2 mls of the strong acid in 100 of water). Shake and allow the mixture to separate, draw off the lower layer into a second separator, wash the residue in the first separator twice with an equal volume of a mixture of alcohol 1 volume, chloroform 2 volumes, drawing off in each case after separation into the second separator. Add to this 15 mls of acidulated water, shake and let separate completely. Draw off the lower layer into a tared beaker. Wash the residual fluid twice with 15 mls each time of the same alcohol-chloroform mixture as before, unite the

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\*Journ. Ind. & Eng. Chem., 1914, 671-2.

chloroformic solutions, evaporate and dry to constant weight at 100° C. and weigh.

**469. A fluidextract** is to be mixed with an equal volume of alcohol and then treated exactly in the same manner as the aliquot above. On the same principle conduct the assay of tinctures or extracts of the drug, dissolving the latter of course in alcohol. Standards should be fixed for the drug and its pharmacopoeial preparations, based on the fact that the drug yields generally 4.75 to 5.25 percent of "podophyllin." Podophyllum Emodi yields a much larger proportion of resin, averaging perhaps as high as 15 to 20 percent.

**470. Standard for podophyllin.** Few of the pharmacopoeias which recognize "resin of podophyllum" or "podophyllin" give any quantitative standard of valuation. The U. S. P. IX requires only that not less than 75 percent of the resin shall be soluble in ether and not less than 65 percent shall dissolve in chloroform—no statement being made as to the quantity of solvent to be used. In other pharmacopoeias it is generally stated that the resin will all dissolve in 100 times its weight of water of ammonia, and in 10 times its weight of alcohol, but that it is only partly soluble in ether or in carbon disulphide—as a rule chloroform is not mentioned, although probably of all solvents it is that which throws most light on the activity of the product.

**470½. The conclusions reached** by H. M. Gordin and C. G. Merrill\* from experimental data were that podophyllum resin should be completely soluble in twice its weight of cold alcohol, that it should contain about 64 percent of ether-soluble and about 74 percent of chloroform-soluble matter and that it should yield about 22 percent of crude picro-podophyllin when assayed in the following manner. Place in a strong bottle of about 200 mls capacity 5 gm. of podophyllum resin with 10 gm. of freshly slaked lime, cork securely and weigh the whole. Remove the cork and place the bottle in a water bath heated to 60°—65° C. After 5 minutes pour in 15 mls

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\*Proc. Am. Pharm. Assoc., 1902, 343-8.

of alcohol, cork the bottle, shake well and replace in the water bath where it is to be left 8 hours, taking care to shake the bottle well every few minutes, (after half an hour, every 15 minutes). Then cool the bottle and add 7 mls of chloroform and in addition sufficient of a mixture of alcohol 2 volumes and chloroform 1 volume to make the total liquid weigh 130 gm. Shake well and set aside (24 hours or more) until the liquid is perfectly clear. Transfer to a tared flask an aliquot part, evaporate, dry and weigh the residue as crude picropodophyllin.

## RHAMNUS BARKS

471. **The several species of *Rhamnus*** which have laxative or cathartic properties—*R. Frangula*, *R. Purshiana*, *R. cathartica*, *R. californica* and *R. carniolica*—agree in that they contain among their active constituents certain oxymethyl-anthraquinone derivatives, the proportion of which has been assumed to bear some relation to the activity of the drug. With regard to the validity of such assumption see (485). The different species show distinct differences qualitatively as well as quantitatively in the effects they produce. Hence the tests which have been proposed, based on the quantity of anthraquinone derivatives present, are of value only for comparing different samples of the same species.

472. **A general method of assay** involving determination of total oxymethyl-anthraquinone derivatives has been proposed by O. Tunmann\*, and is not without merit. Place in an Erlenmeyer flask 1.6 gm. of the dried drug in fine powder with 100 gm. of a solution of sodium hydroxide (3 percent for *R. Purshiana* and *R. cathartica*, 4 percent for *R. Frangula* and *R. carniolica*), heat to boiling, then shake continuously 10 minutes. Let stand to settle, and when cold filter through a double filter into a separator. Mix the residue with 60 gm. more of the alkaline solution, shake 10 minutes and return to the filter. Add the

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\*Apoth. Ztg., 1915, 493.



filtrate to that previously collected, wash the filtrate with 10 gm. of the alkaline solution and finally with a little water. Add to the united filtrate and washings hydrochloric acid, sufficient to change their color to chrome yellow, followed by 160 gm. of chloroform, and shake the fluids together 30 minutes. After 2 hours, draw off the chloroform through a pledget of absorbent cotton and filter it, avoiding loss by evaporation. Transfer 120 gm. of the clear solution to a clean separator, shake several minutes with 120 gm. of the alkali solution, let separate and draw off the chloroform, which is to be rejected. Filter the alkaline solution through a dry double filter. Transfer 100 gm. of it to a beaker, acidulate it with diluted hydrochloric acid and let stand over night. Collect the precipitate on a tared filter, wash it well with water containing 1 percent of its volume of hydrochloric acid. Dry at 60° C. to constant weight and weigh, to find the quantity of anthraquinone derivatives in 1 gm. of the drug.

## FRANGULA

473. **Assuming (fallaciously)** that the activity of frangula is directly proportioned to its potential content of anthraquinone, J. Warin\* has devised an ingenious colorimetric method for assaying the drug, applicable also to other species of rhamnus. The details are as follows: Macerate 0.5 gm. of the powdered bark with occasional agitation with 50 mls of 0.5 percent solution of sodium hydroxide. Filter the solution, transfer 10 mls of the filtrate to a 100 mil measuring flask and fill to the mark with distilled water. Two 50 mil comparison tubes are provided, the one plain, the other graduated to tenthmils; both are covered with black paper except for longitudinal slits 2 mm. wide on opposite sides of each, so that when the tubes are placed in front of one another with the slits coinciding, light rays will traverse the two tubes in succession. A standard

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\*Journ. de Pharm. et Chim., 1905, 253.

green solution is also provided, prepared by dissolving 1 gm. of metallic nickel in 5 mls of a mixture of nitric acid 1 part, hydrochloric acid 3 parts; water is added (with a little more of the acid if necessary) to make up the solution to 100 mls. The green solution will exactly "neutralize" the pink color of a faintly alkaline solution of 1 mg. of emodin in 100 mls of water.

474. Fill the plain tube with the nickel solution and place in the graduated tube 10 mls of the solution prepared from the drug. Arrange the tubes so that light from a white background may be transmitted through the two in succession. If the drug is of normal strength, the pink color will predominate where the light passes through the two solutions. Gradually add distilled water to the tube containing the frangula solution, stirring with a glass rod after each addition, until a point is reached where the pink tint disappears, the transmitted light appearing quite colorless. Read off the volume of the pink fluid and reckon for each mil 0.1 percent of emodin equivalent in the bark. Should the color of the pink solution be so feeble in the beginning that the transmitted light appears green, a second solution must be prepared, using 1 gm. of the drug, and the result found must be divided by two. As in the case of assays of rhubarb based on content of anthraquinone derivatives, the results do not indicate quantitatively the activity of the drug, and are of little or no value for comparison of barks from different species of *Rhamnus*. See (485).

**RHAMNUS PURSHIANA****(Cascara Sagrada)**

**475. To distinguish cascara from frangula bark**, according to L. Kroeber, shake the powdered drug in the cold several minutes with sodium hydroxide solution. The foam, which persists for some time, is strongly reddish in case of frangula, but weakly brownish in case of cascara. If 1 mil of fluidextract of cascara is mixed with 9 mils of water, and a mixture of 6 mils of water and 0.1 mil of a 20 percent solution of mercuric chloride is added, a voluminous precipitate is thrown down at once. No such reaction occurs in case of a fluidextract of fragula or of the kindred drugs, senna, rhubarb, or aloes.

**476. A standard "manganese number"** for fluidextract of cascara sagrada is suggested by L. E. Westman and R. M. Rowat\*, based on the observation that the bark of Rhamnus Purshiana contains a larger proportion of manganese than is found in other laxative drugs, with exception of frangula. Details of the procedure need not be given here, since the value of the test has not been established beyond question. It may serve particularly to distinguish between the barks of Rhamnus Purshiana and R. californica, the latter of which is stated to be comparatively poor in manganese†

**477. Phenolphthalein**, according to the same authors‡, may be detected in fluidextract of cascara sagrada by the following method. Shake out two or three mils of the acidulated fluidextract with 25 mils of benzene. Wash the benzene several times with water in a separator and then shake it with 5 mils of tenth-normal solution of sodium hydroxide, add exactly 4.75 mils of tenth-normal sulphuric acid and

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\*Journ. Am. Pharm. Assoc., Sept. 1918, p. 763.

†Journ. Am. Chem. Soc., March 1918, 560.

‡Journ. Am. Pharm. Assoc., Sept. 1918, p. 767.

again shake well. Wash the benzene again two or three times with water. The benzene will now contain no appreciable amount of emodin, but it will retain phenolphthalein, if this is present, and will therefore show a pink color on shaking with water containing a little sodium hydroxide.

## RHUBARB

478. **The active constituents** of a number of cathartic drugs—aloes, rhubarb, senna, cascara sagrada, frangula and other species of *Rhamnus*—are intimately related chemically, belonging, as we may say, to the anthraquinone family. It is too much to say that the medicinal activity of the several drugs is directly proportioned to the quantity of anthraquinone compounds they contain, yet for individual drugs it has been assumed that approximately at least such a relationship may be predicated\*. As a rule each of these drugs contains more than one of these compounds, so that comparison of one sample with another can be made only when one of these constituents exerts a preponderating influence with regard to the activity of the drug, or else in case it is possible to treat the several constituents in such a way as to bring them practically all to one chemical composition.

479. **In the case of rhubarb**, it is the latter plan that forms the basis for the assay processes that have been proposed by Prof. Tschirch. By treatment with sulphuric acid the anthraglucosides present can be converted into oxymethyl-anthraquinones, which can be easily isolated by shaking out with ether.

480. **Details of the colorimetric method** of Dr. Tschirch are as follows. Boil 15 minutes under a reflux condenser 0.5 gm. of the drug in fine powder with 50 mls of 5 percent sulphuric acid, allow the mixture to cool and without previous filtration shake out repeatedly with ether (50 mls) until one mil of

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\*The fallacy of this assumption, and so of assay processes based thereon, is shown by the investigations of Tutin and Clewer (485).

the ether gives no red color on addition of a few drops of a dilute solution of caustic alkali. Expel residual ether from the aqueous residue by heat and again boil as before under a reflux condenser, cool and repeat the extraction with ether. Shake the united ether solutions with several successive portions (100 mls) of water containing 5 percent of potassium hydroxide, as long as a red color is imparted to the water, and make up to 500 mls. Of this red solution dilute 100 mls with water to 1 liter, and 350 mls of this solution again to 1 liter. Compare the color of this last solution with that of an alkaline solution of aloë-emodin containing 1 part in 1,000,000. Distilled water only is to be used in making the dilutions since calcium salts cause turbidity.

481. If the color is of the same depth as that of the standard, the rhubarb contains the equivalent of 2.4 percent of oxymethyl-anthraquinone, otherwise the quantity is in direct proportion to the depth of color, so that if the solution requires dilution with 20 percent of its volume of water, we must add to 2.4 percent  $2.4 \times 0.20 = 0.48$ , making altogether 2.88 percent. According to the author, rhubarb should show by this test not less than 2.8 percent of oxymethyl-anthraquinones.

482. **Gravimetric method** of Tschirch and Edner\*. This depends on the fact that oxymethyl-anthraquinone forms with para-diazonitroaniline an insoluble compound representing 35.6 percent of its weight of chrysophanic acid. The assay is made in the following manner: Boil 0.5 gm. of the powdered drug with successive portions of a diluted alcoholic solution of potassium hydroxide until completely exhausted. Distil off the alcohol, dilute the residue with water, acidulate with hydrochloric acid, collect the resulting precipitate on a filter, wash with acidulated water and dry. Transfer the filter containing the precipitate to a soxhlet and extract several hours with chloroform, which dissolves the anthraquinones. Distil off the chloroform and dis-

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\*Arch. d. Pharm., 1907, No. 150.

solve the residue by aid of heat in 10 mls of 5 percent solution of sodium hydroxide and dilute with 50 mls of water. Add 20 mls of a para-diazonitroaniline solution, prepared by shaking together in a 500 ml stoppered flask 25 mls of water, 5 mls of concentrated sulphuric acid, and 5 gm. of para-nitroaniline, then adding 100 mls of water, followed by a solution of 3 gm. of sodium nitrate dissolved in 25 mls of water, and finally water sufficient to make 500 mls. (This reagent must be kept protected from light.)

483. Add next, drop by drop, hydrochloric acid until the solution is decolorized and the coloring matter completely precipitated. Make sure that the solution has an acid reaction, then set the mixture aside for several hours to allow the precipitate to subside, finally collect this on a tared filter, wash until the washings cease to show an acid reaction, dry at 70° C. to constant weight and weigh. Multiply the weight of the precipitate by 0.356 to find the quantity of oxymethyl-anthraquinones, reckoned as chrysophanic acid, in the sample taken for assay.

484. **Discrimination of rhapontic** from genuine rhubarb may be made by the following method, from Caesar and Loretz's Report, 1906\*. Boil 10 gm. of the powdered drug 15 minutes with 50 mls of diluted alcohol, filter and wash the residue on the filter with 20 to 25 mls of diluted alcohol. Evaporate the filtrate and washings until alcohol is completely driven off, add water to bring the volume to 10 mls, cool and shake with 10 to 15 mls of ether. If the rhubarb is genuine, the liquid remains clear even after standing 24 hours. In the case of rhapontic rhubarb, colorless needle-shaped crystals of the glucoside **rhaponticin** appear after a time, which, if collected on a filter, washed with water and dried, produce with sulphuric acid a purple red color, changing soon to orange.

485. **The most recent investigation** of the constituents of Chinese rhubarb, that of F. Tutin and H. W. B. Clewer†, makes it evident that none of the

\*Pharm. Ztg., 1906, No. 75, 830; see also Schweitz. Woch. Pharm., 43, 253, 1-4.

†Journ. Chem. Soc., 1911, Trans. 946-67.

proposed methods of assay are of much value as indicating the medicinal activity of any sample of the drug. The authors report that they found in the drug examined, besides constituents of no importance in this connection, rhein, 0.12 percent; emodin, 0.78; aloe-emodin, 0.16; emodin monomethyl ether, 0.22; chrysophanic acid, 0.49; crystalline mixture of glucosides of anthraquinone derivatives, 2.0; nonglucosidic resin, 10.4; tannin, 0.52. The only constituents belonging to the anthraquinone group which had purgative activity were demonstrated to be aloe-emodin and chrysophanic acid, whereas the nonglucosidic resin is more active than either of the constituents just named and so constitutes by far the most important "active principle" in the drug, being responsible for more than 95 percent of its medicinal efficiency. The authors did not succeed in isolating from it however, anything of chemical individuality.

## SCAMMONY ROOT

486. **The active constituent** of scammony is an ether-soluble resin. The methods of assay described under Jalap are equally applicable to this drug, but the resin extracted should be shown to be soluble at least to the extent of 95 percent in ether, whereas the greater part of the resin from jalap is insoluble in that menstruum. The U. S. P. IX adopts without modification the assay process for jalap. See (460) and (460½). There should be an added requirement regarding ether-solubility. The standard of the U. S. P. IX for total resin is not less than 8 percent.

487. **The French Codex** provides an assay for scammony (the gum resin) which determines the proportion of ether-soluble resin, substantially as follows: Triturate thoroughly 1.5 gm. of scammony with an equal weight of fine sand, previously ignited. Transfer 2 gm. of the powder to a suitable flask with 50 mls of ether. Macerate with occasional shaking during 12 hours. Decant the ether upon a plain filter and collect the filtrate in a tared Erlenmeyer

flask; add to the residue 25 mls of fresh ether, shake well, let settle and decant upon the same filter as before. Repeat this procedure once more, then distil off the bulk of the ether and dry the residue in the Erlenmeyer at 100° C., by aid of an air current, to constant weight and weigh. Standard not less than 43.3 percent resin.

488. The same process may be employed for scammony root, using 5 gm. of the finely powdered drug. The extraction can be advantageously made in a soxhlet apparatus.



## Chapter V

### Vegetable Drugs, Non-Potent

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#### ACACIA

489. **Gums are notoriously** of variable composition, so that exact quantitative determinations may be said to be out of the question. In a general way they are separated from aqueous solutions in proximate organic analyses by precipitation with alcohol. Arabin is precipitated in presence of dextrin and similar mucilage-forming bodies by an alkaline copper solution (e. g. Fehling's solution), and this has been made the basis of an assay process which gives useful although not exact results.

490. **The best assay process yet devised** is probably that of C. E. Waters and J. B. Tuttle\*. One gm. of the sample (gum arabic) is dissolved in water sufficient to make 200 mls. A copper reagent is prepared by dissolving 50 gm. of copper acetate in water, adding water of ammonia in excess, making up with water to 480 mls and adding alcohol sufficient to make the volume, when cooled to standard temperature, 1000 mls. For the assay, transfer to a beaker 50 mls of the solution of gum, add an equal volume of 95 percent alcohol and then, with constant stirring, 25 mls of the copper reagent. Collect the precipitate on a tared filter, wash it with 50 percent alcohol containing ammonia, then with 75 percent, and finally with 95 percent alcohol, dry to constant weight at 105° C. and weigh. Ignite the dried precipitate in a porcelain crucible and weigh the residue of copper oxide. Subtract this from the gross weight of the dried precipitate to find the weight of arabin. [It would seem advisable to dissolve the copper oxide in nitric acid, evaporate the solution and ignite the residue before weighing. The weight of the copper

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\*Journ. Ind. and Eng. Chem., 1916, 413-6.

oxide should be multiplied by a factor (tentatively 0.82) before subtracting from the weight of the precipitate. Ed.].

## ASAFETIDA

491. **The drug is one liable to contain** a large proportion of accidental impurities, owing to carelessness in collecting as well as deliberate sophistication. Pharmacopoeial requirements are generally limited to a maximum of ash (in U. S. P. IX, 15 percent) and a minimum of alcohol-soluble constituents (in U. S. IX, 60 percent, or for the powdered drug, 50 percent). Obviously such "standards" mean very little—nothing whatever as to the medicinal value of any sample. It is contended by many that genuine asafetida of good quality often contains fully 50 percent of alcohol-insoluble matter, so that the U. S. P. requirement is too stringent.

492. **An arbitrary requirement** of the U. S. Customs Department is that the drug shall have a minimum "lead number," i. e. that the ether-soluble resins shall take up a stated minimum of lead from a hydro-alcoholic solution of the acetate to form alcohol-insoluble compounds\*. The test has no scientific basis or value. On the other hand, there is reason to believe that the proportion of sulphur contained in the essential oil obtained from the drug by distillation gives a trustworthy indication of the value of the sample.

493. **Assay process of Harrison and Self†.** From 15 gm. of the drug, distil off the volatile constituents in a current of steam. Separate the oil which rises to the surface, shake out the remainder of the oil from the aqueous solution with petroleum benzin of low boiling point, evaporate the solvent and add the residue to the separated oil. Determine sulphur in the oil in the following manner: Weigh in a 150 mil flask, about 0.5 gm. of the oil, fit the flask

\*Harrison and Self, *Pharm. Journ.*, 1913, 218; J. R. Rippetoe, *Am. Journ. Pharm.*, 1913, 199; E. J. Parry, *Chem. & Drugg.* 1913, 34.

†*Pharm. Journ.*, 1912, 205; *Yearbook of Pharmacy*, 1912, 417.

by a ground joint to a vertical condenser, add 5 mls of water followed by 5 mls of nitric acid, start reaction if necessary by applying a gentle heat. When the reaction moderates, add through the condensing tube 3 gm. of potassium bromide in powder, boil the mixture 10 minutes, cool and add in the same manner 5 gm. of sodium hydroxide, dissolved in a little water. Transfer the contents of the flask to a platinum dish, evaporate to dryness and ignite. Dissolve the residue in water, transfer to a beaker and remove nitric and nitrous acids by evaporation with hydrochloric acid. Then precipitate the sulphuric acid with barium chloride in the usual manner. Each gm. of barium sulphate corresponds with 0.13738 gm. of sulphur in the oil (a blank test must be made with the reagents used, and any barium sulphate resulting must be deducted from that taken to represent sulphur).

494. In the samples of asafetida examined by Harrison and Self, the proportion of volatile oil ranged from 5 to 20 percent, averaging 11.88; the proportion of sulphur in the oil ranged from about 17 to 38 percent (in one sample out of 26 it was 8.9 percent); average 22.12. A reasonable minimum requirement of volatile oil would be 5 percent, and of sulphur (contained in the volatile oil) 1.1 percent of the weight of the drug.

## ASPIDIUM (MALE FERN)

494a. **For purposes of standardization** the active constituent of aspidium may be assumed to be crude filicic acid, although this is admitted to be quite variable in therapeutic activity. The crude drug is to be judged by its appearance. Old samples which have lost their green color are not fit for medicinal use. The drug may be assayed by extracting from it its oleoresinous constituents and determining in this extract the crude filicic acid.

494b. **Assay of the oleoresin.** This preparation is one which has been often grossly adulterated, particularly by addition of castor oil. The charac-

ters of a genuine product are\*: sp. gr., 1.018 to 1.052 at 15° C.; refractive index, 1.4995 to 1.5157 [at 20° C. (?)]; saponification value, 227 to 259; unsaponifiable, 4.1 to 6.7 percent; insoluble in petroleum ether, 3.2 to 14.8 percent. (Note that castor oil is insoluble in petroleum ether); crude filicic acid (by assay of the Swiss Pharmacopoeia) 19.3 to 28.0 percent. This last serves as the basis for a provisional standardization, which enables us at least to guard against gross adulteration.

494 c. The method is a modification of that of Fromime. The oleoresin, which often contains much insoluble matter, is to be triturated to form a homogeneous mixture, of which about 5 gm. is to be taken for the assay. Weigh this accurately, dissolve it in 40 mls of ether in a 200 ml Erlenmeyer flask. Add 100 mls of a 3 percent solution of barium hydroxide and shake the mixture vigorously during 5 minutes. Transfer to a separator and after 10 minutes draw off through a filter 87 mls of the aqueous solution. Add to this sufficient hydrochloric acid to render it distinctly acid and shake out the liberated filicic acid with ether (30, 20 and 15 mls). Filter the ether through a pledget of absorbent cotton, evaporate off the solvent, dry the residue to constant weight at 100° C. and weigh as crude filicic acid. According to A. Goris and M. Voisin† the results of the assay conducted in this manner are too high. They direct to drive off the dissolved ether from the aqueous solution, causing separation of certain impurities which are removed by filtration before adding the hydrochloric acid. It is not claimed, however, that the filicic acid obtained by this procedure is of uniform anthelmintic activity, and under the circumstances it seems best to follow the method of the Swiss Pharmacopoeia without modification.

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\*E. F. Harrison and P. A. W. Self, Yearbook of Pharmacy, 1913, 496. Additional references: E. T. Parry, Pharm. Journ. 1911, 778, from the Analyst; A. J. Du Mez, Philipp. Journ. Sci., 1915, 8.

†Bull. Sci. Pharm., 1913, 19, 705.

## BENZOIN

495. **The requirement of the U. S. P. IX** for Benzoïn (either Sumatra or Siam) is that it shall yield to warm carbon disulphide 12.5 percent of its weight of a residue which responds to the official tests for benzoic acid. Sumatra benzoïn yields to **alcohol**, it is stated, not less than 75 percent of its weight; Siam benzoïn not less than 90 percent. The method of making the test is not prescribed. Sumatra benzoïn yields not more than 2.5 percent of ash; Siam benzoïn not more than 2 percent.

496. **Assay method of T. T. Cocking and J. D. Kettle\***, for determination of (1) alcohol-soluble constituents, (2) total balsamic acids, (3) cinnamic acid by bromination and (4) benzoic acid by difference. The sample is first dried over sulphuric acid in vacuo to determine moisture. Five gm. of the dried powder are exhausted with alcohol in a soxhlet apparatus, the residue is dried and weighed, the alcohol-soluble substances being determined by difference. The alcoholic solution is saponified in the usual manner with excess of potassium hydroxide, the alcohol is evaporated off, the mass is dissolved in 100 mls of water and the solution rendered slightly acid with hydrochloric acid. Five gm. of light magnesium oxide and 20 mls of xylene are added, and the mixture is boiled one hour under a reflux condenser. The aqueous solution is cooled and filtered, the filter and residue are returned to the flask and boiled again with 100 mls of water, the solution cooled and filtered and the extraction of the residue with water is repeated once more. The aqueous solutions are mixed, shaken out once with ether to remove impurities, then rendered acid with hydrochloric acid and shaken out repeatedly with ether. The ether is evaporated off by a gentle heat, and the residue of benzoic and cinnamic acids is dried in vacuo over sulphuric acid and weighed.

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\*Yearbook of Pharmacy, 1914, 357-60.

497. **Cinnamic acid** in the mixture is determined by adding decided excess of a 5 percent solution of bromine in carbon tetrachloride, allowing to stand over night, then evaporating off solvent and excess of bromine, treating the residue several times with a little ether and evaporating, finally drying the residue in vacuo over sulphuric acid and weighing. Each molecule of cinnamic acid takes up two atoms of bromine. Multiply the gain in weight by 0.9263 to find the quantity of cinnamic acid.

## CAPSICUM

498. **The pungency of capsicum** has been demonstrated to be due to a crystallizable compound, capsaicin, to which has been assigned the empirical formula  $C_{18}H_{28}O_3N^*$ . This is present in very small proportion; in a selected sample of African capsicum *C. fastigiatum*, E. K. Nelson found only 0.14 percent†. Its isolation involves laborious and complicated manipulations, so that a practicable assay process is not as yet available. The "standard" of U. S. P. IX, which requires merely that capsicum shall yield at least 15 percent of non-volatile extractive soluble in ether, is wholly arbitrary and really serves no useful purpose.

499. **Biological (organoleptic) test for capsicum.** It is quite possible to form a reasonably exact judgment of the "strength" of a sample of the drug by the simple expedient of testing its pungency. W. L. Scoville proposes the following practical method. Macerate 0.1 gm. of ground capsicum over night in 100 mls of alcohol; shake well and filter. Add this tincture to sweetened water (10% sugar) in such proportion that a distinct but weak pungency is perceptible to the tongue or throat. According to Scoville official capsicum will respond to this test in a dilution of 1:50,000. He found the Mombassa chilles to

\*Thresh, in Pharm. Journ. and Trans., 1876, 7, 21, 259, 473; Micko, in Zeitsch. f. Unters. d. Nahr. u. Genussm., 1898, 813; 1899, 411.

†Journ. Ind. and Eng. Chem., 1910, 419.

test from 1 : 50,000 to 1 : 100,000; Zanzibar chillies, 1 : 40,000 to 1 : 45,000; Japan chillies 1 : 20,000 to 1 : 30,000. Nelson found that a single drop of a solution of capsaicin in alcohol 1 : 1,000,000, applied to the tip of the tongue produced a distinct impression of warmth.

500. **To give definiteness** to the test, a quantity of the solution not less than 2 mils (better 4 mils) should be used, and this should be given a definite time (e. g. 30 or at most 60 seconds) for action. Under such conditions the limit of dilution would no doubt be considerably greater than that reported by Scoville. However, individual susceptibility must vary greatly, so that to be of any value the test would have to be a comparative one, and it would be necessary to have a standard for the comparison.

501. **To detect capsicum** as an adulterant in beverages containing ginger, Nelson directs\*, as a modification of the procedure of Garnett and Griert†, to evaporate off alcohol if present (from 100 mils of the sample), shake out the residue with ether, which will extract the pungent principles of both ginger and capsicum, treat the residue in a porcelain dish with 10 mils of double-normal alcoholic potassium hydroxide solution and evaporate to dryness on a steam bath. Add about 0.006 gm. of powdered manganese dioxide with 5 to 10 mils of water, and continue heat 20 minutes, or until volatile oils are dissipated. Cool, acidify with dilute sulphuric acid and extract at once with petroleum benzine. Evaporate the solvent in a small crucible, keeping the residue within as small an area as possible, heat 5 minutes on a steam bath. The prescribed treatment destroys the pungency due to ginger but does not affect the capsaicin that may be present. Hence by applying the tip of the tongue to the residue, often scarcely visible, one learns whether or not capsicum has been used.

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\*Loc. cit., p. 421.

†Pharm. Journ. and Trans., (3), 12, 721.

## GINGER

**502. The researches of J. C. Thresh (1880-1885)** resulted in descriptions of a number of active principles of pungent drugs—among them ginger. The name given to this principle, gingerol, was a confession that it was not a definite chemical compound. Further study was made about 1907 of this “principle” by James Grier and H. Garnett, who demonstrated the fact that it consisted only in part of a compound (or compounds) of a phenolic character. Recent studies (1917) by A. Lapworth\* have added materially to our knowledge of the nature of this substance, from which he has been able to separate a pungent principle whose constitution has been determined and verified by synthesis.

**502½.** E. K. Nelson† assigns to gingerol, which he finds to be identical in composition with paradol, the pungent constituent of grains of paradise (*Amonum Melegueta*, Roscoe), the formula  $C_{17}H_{24}O_2(OCH_3)_2 = 322.24$ .

**503. Approximate Assay.** For practical purposes the process of H. Garnett and J. Grier‡ for assay of ginger may be considered satisfactory. Exhaust the finely powdered drug, preferably by hot repercolation, with ether, distil off the solvent, boil the residue with successive portions of petroleum benzin as long as anything is dissolved, and shake out the united benzin solutions with three successive portions of 60 percent alcohol. Wash the alcoholic solution once with petroleum benzin to remove traces of impurities, and shake out the gingerol with several portions of ether. Evaporate the ether, dry the residue half an hour at 80° C. and weigh as gingerol. The purity of the gingerol may be established by dissolving it in cold 1 percent aqueous solution of potassium hydroxide. The yield of gingerol according to

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\*Pharm. Journ., [4], 44, 201.

†Journ. Am. Chem. Soc., 1917, 1466-9.

‡Yearbook of Pharmacy, 1909, 344-6.



these authorities is, from Jamaica ginger, 1.1 percent; from African ginger, 2.0 percent. These figures however, were based on insufficient data, and a standard cannot as yet be fixed.

504. Possibly an assay could be made by extracting the ginger first with petroleum benzin and afterwards with ether. If the petroleum benzin is found to extract some gingerol, this can be recovered by shaking out with 60 percent alcohol as in the foregoing paragraph.

505. The U. S. P. IX requires that ginger shall yield to alcohol (?) not less than 4 percent of extractive. The method of extraction is not specified and the language used is ambiguous ("Ginger yields not less than 4 percent of an extract soluble in alcohol").

506. The requirement of the British Pharmacopoeia is explicit. When 5 gm. of powdered ginger are shaken with 100 mls of alcohol (90 percent) occasionally during 24 hours and filtered, 20 mls of the filtrate yield on evaporation not less than 0.050 gm. of residue (i. e. 5%) dried at 100° C. There is an added requirement that when 5 gm. are similarly treated with 100 mls of water, 20 mls of the filtrate shall yield not less than 0.085 gm. of residue dried at 100° C. This is to exclude a drug from which a portion of the active principle has been extracted, and the U. S. P. IX has practically the same requirement. Another requirement of the U. S. P. IX is that the drug shall yield not less than 2 percent of non-volatile extract soluble in ether, language naively ambiguous, as in the case of alcohol-soluble extract. It has been shown\* that the extractive taken out by strong alcohol from ginger is practically all soluble in ether, so that it seems hardly necessary to provide a separate standard for an ether extract.

507. The U. S. P. IX provides no standard for the **fluidextract** or for the **oleoresin** of ginger, but it does require that the **tincture** shall contain not more than 2 percent (by weight) of extractive, of which not

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\*C. W. Harrison and A. L. Sullivan, Journ. A. O. A. C., 1915, 506.

more than 15 percent shall be soluble in a specified volume of cold water. The object of the test would be equally well accomplished by fixing a maximum specific gravity to insure that the alcohol used as the menstrum shall be of official strength. The directions with regard to testing the water solubility of the extract are too vague to be of any value.

508. **The most important requirement** of the U. S. P IX is that the tincture shall be free from capsicum or similar pungent substitutes. These are to be detected by the test described in (501). The same test might well be given for the fluidextract and even the oleoresin of ginger.

## HUMULUS

**509. Determination of the resinous constituents** of hops is made by L. Briant and C. S. Macham in the following manner. Place in a soxhlet apparatus 4 gm. of the sample and extract it 24 hours with petroleum ether, boiling at about 50° C. Evaporate the solution, dry the residue in the steam oven and weigh as soft resins. Extract the residue in the soxhlet apparatus with "ordinary" ether 12 hours, evaporate and dry and weigh the residue as hard resins. Until a more exhaustive study shall have been made of the numerous constituents of hops, however, we are without any scientific basis for standardization of the drug.

## LICORICE

**510. The active constituent of licorice** is familiarly known as glycyrrhizin. More correctly it is designated glycyrrhizic acid. It is a crystallizable substance, and forms with certain bases readily crystallizable salts of perfectly definite composition. It is rather freely soluble in pure water, but is only sparingly soluble in water containing sulphuric acid. The precipitate thrown down by the acid is by no means pure glycyrrhizic acid, being contaminated especially with an intensely bitter principle, which is particularly unfortunate since the chief use of licorice is to mask bitter and disagreeable tastes.

**511. The assay process of Hafner\*** aims to isolate and determine the glycyrrhizic acid, and is conducted as follows, taking the extract of licorice as a starting point. Put into a suitable flask 10 gm. of the extract in coarse powder, add 200 mls of alcohol and 25 mls of normal sulphuric acid and shake the mixture occasionally during several hours, a gentle heat being advantageously employed. Filter the fluid, wash the residue with alcohol until the filtrate

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\*Zeitschr. Oestr. Ap. Ver., 1899, 542.

passes colorless, add an equal volume of water and then water of ammonia to faint alkalinity. Evaporate off the alcohol and make up the aqueous solution to 100 mls, having cleared up any turbidity by addition of a few drops of ammonia water. Filter and to 80 mls of the filtrate add, with constant stirring, diluted sulphuric acid to complete precipitation. Collect the precipitate on a filter and wash with highly dilute sulphuric acid (2 to 3 percent) until the filtrate passes colorless. Dry the filter and contents as well as possible in a vacuum over sulphuric acid at room temperature, transfer to a beaker and extract on a water bath with successive portions of acetone as long as color is extracted.

511½. Evaporate the united acetone solutions for glycyrrhizic acid in a high beaker with addition of excess of precipitated barium carbonate, mix the residue with 200 mls of hot water, gradually added, decant the clear solution of barium glycyrrhizate into a 500 ml flask and wash the residue with successive portions of hot water until 500 mls of solution are obtained. Cool, make up the volume to exactly 500 mls; after subsidence of any barium carbonate that may have come over in decanting, measure 125 mls of the clear solution, filtered if necessary; evaporate and dry to constant weight. Multiply the weight by 0.405 to find the amount of pure glycyrrhizic acid, or by 0.428 for the ammonium glycyrrhizate represented in 1 gm. of the sample. Otherwise, ignite the precipitate with sulphuric acid (twice) to convert it into barium sulphate, multiply the weight of this by 1.290 to find weight of pure glycyrrhizin or by 1.35 to find weight of ammonium glycyrrhizate, corresponding with 1 gm. of sample.

## MUSTARD

512. Nearly all of the numerous assay processes for volatile oil of mustard depend on reaction of the allyl isothiocyanate in the oil with ammonia, producing a compound which gives up its sulphur readily to silver nitrate. Either the silver sulphide itself is collected and weighed (1 gm. = 0.4007 allyl isothiocyanate) or, more commonly, a definite volume of tenth-normal silver nitrate is taken and the excess of silver, after reaction with the sulphur compound, is determined either volumetrically or gravimetrically, from which is deduced the quantity of sulphur and thence that of the allyl isothiocyanate in the sample taken. Each mil of the volumetric silver solution corresponds with 0.004956 gm. of allyl isothiocyanate.

513. **Assay of powdered mustard seed** may be made either by the method of D. Raquet\*, or by that of H. Penau†. The former is carried out as follows: Put into a 250 mil distilling flask 5 gm. of powdered mustard with 100 mls of water and 20 mls of 90 percent alcohol, cork the flask and keep at a temperature of 30° to 35° C. by aid of a water bath six hours. Distil slowly over a glycerin bath into a 100 mil measuring flask containing 10 mls of water of ammonia, letting the delivery tube from the condenser dip below the surface of the ammonia. When about 50 mls of distillate have come over, add to the receiver 20 mls of tenth-normal silver nitrate V. S. and continue the distillation until the receiver is filled to the mark. Heat the mixture under a reflux condenser one hour at a regulated temperature of 80° to 85° C. When cold, readjust to a volume of 100 mls, shake, filter, rejecting the first 25 mls of filtrate, then collecting exactly 50 mls. To this add 5 to 6 mls of nitric acid, sp. gr. 1.39, and titrate excess of silver with tenth-normal potassium sulphocyanate, using solution of ferric alum as indicator. Multiply the

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\*Repertoire Pharm., 1912, 24, 145.

†Journ. Pharm. Chim., 1912, 6, 160.

volume in mls of the sulphocyanate solution consumed by two, subtract from 20 and multiply the remainder by 0.004956 to find the weight of the allyl isothiocyanate in the sample taken. F. Wehrmann\* after a careful study of the various assay methods that have been proposed, accepts the foregoing as that most satisfactory, but H. Penau† after a similar study declares the method untrustworthy, maintaining that the determination of residual silver should be made by the potassium cyanide method.

514. **The method of Penau** is as follows: Put into a dry flask 5 gm. of powdered mustard, add 100 mls of water, cork well and let stand six hours, (according to Raquet, it is important that some alcohol be present during this maceration.) Add 20 mls of alcohol and 20 mls of poppy seed oil. Connect the flask with a condenser and distil very slowly over a glycerin bath into a receiver containing 5 mls of water of ammonia, sp. gr. 0.925, and 5 mls of water, letting the delivery tube reach below the surface of this mixture, until the volume of fluid in the receiver reaches 100 mls. Add to this fluid 20 mls of tenth-normal silver nitrate, cork the flask and set by in the dark 24 hours. Remove the precipitate by filtration, render combined filtrate and washings acid with nitric acid, precipitate the excess of silver with hydrochloric acid, collect, wash and dry the precipitate in the usual manner. Subtract the weight of the precipitate from 0.28668 and multiply the remainder by 0.3458 to find the weight of the allyl isothiocyanate in the sample taken. If a volumetric determination of the excess of silver is preferred, it should be made by titration of the ammoniacal solution, to which has been added a little potassium iodide, with tenth-normal potassium cyanide solution.

515. **It is possible** that in some cases the whole of the oil of mustard is not shown where the distillation is carried only as far as directed in the foregoing assay processes. It is safe therefore, after

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\*Arch. Pharm., 1915, 306.

†Journ. Pharm. Chim., 1912, 6, 160-4.

distilling to 100 mls, to change the receiver for another containing 5 mls of water of ammonia and 5 mls of tenth-normal silver nitrate and distil into this 15 to 20 mls of fluid, treating this second distillate in the same way as the first.

**516. Assay of volatile oil of mustard** or of spirit of mustard. The same methods as those for assay of mustard, *mutatis mutandis*, are employed for the oil or spirit. The preliminary maceration is of course unnecessary as is the distillation. About two gm. of the oil are weighed accurately, made up to 50 mls with alcohol and an aliquot of 5 mls of the solution is taken for the assay. In case of a spirit of mustard, a quantity judged to contain not to exceed 0.2 gm. of the oil is taken. In either case, 50 mls of tenth-normal silver nitrate solution is added and the mixture is heated on a water bath under a reflux condenser one hour. When cool the solution is filtered and the excess of silver in the filtrate and washings is determined as a basis for calculating the quantity of allyl isothiocyanate in the sample under examination. The details obviously, after the initial steps, are the same as in the assay processes described above (513) and (514). The U. S. P. IX, B. P. 1914 and P. G. all adopt this plan, using the Volhard method of determining excess of silver. Each ml of tenth-normal silver nitrate solution corresponds with 0.004956 gm. of allyl isothiocyanate.

**517. Among the methods** for determining allyl isothiocyanate, in which a silver salt is not used, may be mentioned (1) that of O. Foerster\*, in which mercuric oxide replaces the silver salt, the mercuric sulphide being separated and weighed as a basis for calculating the result (not favorably considered at the present time); (2) that of B. Grützner†, in which the oil is treated with ammonia during 12 hours, the solution heated on a water bath until the ammonia is driven off, sodium peroxide (free from sulphate) is added, by which the sulphur in the oil is oxidized to

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\*Chem. Zeit., 1895, 19, 1423-5.

†Arch. Pharm. 1899, 237, 185-9.

sulphuric acid, and this is finally determined gravimetrically or volumetrically (perhaps good as a confirmatory test), and (3) that official in the Swiss Pharmacopoeia, in which the oil is diluted with alcohol and treated with ammonia, and the resulting thiosinamin is dried and weighed (not to be recommended.)

## PERU BALSAM

518. **The leading pharmacopoeias agree** in giving importance to the proportion of crude cinnamein contained in Peru Balsam, the U. S. P. IX making the minimum requirement 50 percent (maximum 56 percent), the British 57 percent, the German 56 percent, the Swiss 60 percent. The identity of the cinnamein is in a measure assured by the requirement that its saponification value shall be at least 235. Determination of the cinnamein is best made as prescribed in the British Pharmacopoeia as follows: Dissolve 1 gm. of the sample in 30 mls of ether in a separator, shake out with two successive portions of half-normal sodium hydroxide solution (20 and 10 mls) which are transferred to a second separator and shaken out with 10 mls of ether. Reject the alkaline solution and add the ether to that in the first separator. Wash the ether with 2 successive portions (5 mls) of water. Transfer the ether to a tared beaker, rinse the separator with ether (5 and 5 mls), which is also transferred to the beaker, evaporate until odor of ether disappears, add 1 ml of alcohol and evaporate to dryness, heat at 100° half an hour, cool and weigh as cinnamein.

519. **The method of the U. S. P.** as also that of the G. P. differs from the foregoing in that a fixed quantity (volume) of ether is used in the extraction and that one aliquot portion (volume) of the ether is evaporated for determination of the cinnamein. As pointed out by Lehmann and Mueller\* the aliquot is inexact, but results may be considered sufficiently precise for the purpose of the test. It is better, however, to select a method like that of the B. P. which is

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\*Arch. d. Pharm., 1912, 1, 1.



not open to such criticism, particularly if the permissible percentage of cinnamein is to have so limited a range (50–56 percent).

520 The requirements of the U. S. P. IX are that the "acid number" of the balsam (576, foot note) shall be not less than 56 nor greater than 84, and that the "saponification value" (144) of the cinnamein shall be from 235 to 238.

521. **Peruviol has been proposed** by Dodge and Sherndal\* as a basis for valuation of Peru balsam. The proportion is small but none at all is likely to be found in an imitation balsam. It is characterized not only by its distinctive odor but by its high optical activity, viz.  $+13^{\circ}$ . It is determined by heating one hour on a water bath 25 gm. of the sample with 20 gm. of a 25 percent solution of potassium hydroxide and distilling by a current of steam to obtain 300 mls of distillate. The oil rises to the surface and its volume can be read off in a cassia flask, the yield being, from 25 gm. of genuine Peru balsam, about 3.7 to 4.7 mls. The specific rotation of the crude peruviol obtained in this manner is about  $+10^{\circ}$ . It is soluble in 70 percent alcohol, and shows an iodine value of about 116.

## STORAX

522. **Cinnamic acid in storax** is determined approximately by the following method, official in the British Pharmacopœia†. Dissolve 2.5 gm. of the sample in 25 mls of half-normal alcoholic potassium hydroxide solution, boil for one hour under a reflux condenser, neutralize with half-normal sulphuric acid, remove the alcohol by evaporation and dissolve the residue in 50 mls of water. Shake this solution with 20 mls of ether; after separation draw off the ethereal layer, wash this with 5 mls of water which is to be added to the aqueous solution, rejecting the ether. Acidify the aqueous solution with diluted sulphuric

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\*Journ. Am. Pharm. Assoc., Oct. 1915, 1222–6.

†As suggested by C. A. Hill and T. T. Cocking, Chem. and Drugg., 1912, 80, 412.

acid and shake out with four successive portions (20 mls) of fresh ether. Wash the mixed ethereal solutions with a few mls of water, transfer to a flask and distil off (or evaporate) the ether. To the residue add 100 mls of water and boil vigorously 15 minutes under a reflux condenser. Filter the solution while hot, cool to 15.5° C., and collect on a tared filter the crystals of cinnamic acid that have separated. Repeat the extraction of the residue with boiling water at least three times, or until no more crystals form. Press the filter and contents between folds of filter paper, dry in a desiccator over sulphuric acid and weigh. Add 0.03 gm. as correction for solubility. The corrected weight should not be less than 0.5 gm.

## TOLU BALSAM

523. **The requirements of U. S. P. IX** for tolu balsam are that the "acid number"\* shall be not less than 112 nor more than 158, and the "saponification value" (144) shall be not less than 154 nor more than 220. Similar requirements are found in other up-to-date pharmacopœias, and considering the nature of the product are perhaps sufficiently exacting.

524. **T. Delphin† recommends** the following more complete assay of the drug. The sample is dissolved in ether and shaken with normal alkali. The separated ether is evaporated and the residue is dried and weighed as cinnamein. The alkaline solution is precipitated with sodium bicarbonate; the precipitate, consisting of resin esters, is collected, washed, dried and weighed. The filtrate and washings are acidulated with hydrochloric acid and the

\*The acid number of a resin is the number of milligrams of potassium hydroxide required to neutralize the acids present in 1 gm. of the resin, phenolphthalein being used as indicator. Weigh accurately about 2 gm. of the resin, dissolve this in alcohol (25 mls), add a few drops of the indicator and titrate with half-normal potassium hydroxide. Multiply the number of mls of volumetric alkali consumed by 28.055 and divide the product by the weight in grammes of the sample taken.

†Svensk Farm. Tidskr., 1907 Nos. 3, 4 and 5. Pharm. Ztg., 1907, 407.

resulting precipitate of resin acids is collected, washed, dried and weighed. The filtrate and washings from these are shaken with ether and the ether solution is titrated with volumetric alkali to determine combined cinnamic and benzoic acids. Finally cinnamic acid may be determined by bromination as in assay of benzoin (497).

## WILD CHERRY

525. **The medicinal value** of wild cherry bark depends chiefly on the hydrocyanic acid which it yields when treated with water. An assay process based on this principle has been employed by A. R. L. Dohme,\* and in a simplified form, has been recommended by A. B. Stevens†. The procedure of the latter is as follows: Place in an 800 mil flask 10 gm. of the ground bark with 100 mls of water. Cork securely and let macerate 24 hours. Place the flask in a water bath and connect with an efficient condenser. Arrange a second flask to deliver steam into the bottom of the first. Bring the water bath to boiling and then deliver a brisk current of steam through the contents of the flask for 20 minutes.

526. The delivery tube of the condenser is to be carried to the bottom of a small flask in which has been placed 10 mls of a tenth-normal solution of silver nitrate and 20 mls of distilled water. Any uncondensed vapor is carried from this flask by a tube reaching to the bottom of a second flask charged with a silver solution similar to that in the first flask. The hydrocyanic acid is absorbed by the silver solutions, which, at the close of the process are united, and the residual silver is determined with a tenth-normal solution of potassium sulphocyanate by Volhard's method. Subtract the quantity in mls of sulphocyanate consumed from the total quantity of silver solution, and multiply the remainder by 0.02802 to find the percent of potential hydrocyanic acid in the sample.

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\*Pharm. Rundsch., XIII, 260.

†Proc. Am. Pharm. Assoc., 1896, p. 216.

527. **The plan adopted by Dr. Dohme** differed from the foregoing in the use of tenth-normal solution of potassium hydroxide to receive the hydrocyanic acid, the quantity of which is determined by titration with tenth-normal silver nitrate, of which 1 mil corresponds with 0.005404 gm. of HCy. The method seems to give somewhat higher results than the preceding, possibly from the more certain capture of the vapor of hydrocyanic acid by the alkaline solution.

527½. **Results of assays** made by Dr. Stevens show that the bark of the root contains the largest proportion of the glucoside yielding hydrocyanic acid, the bark of young trees more than that of old ones, and bark from the twigs more than that from the trunk of the tree. The percentage of hydrocyanic acid to be expected from freshly gathered bark would be, for the bark of the root, 0.2 to 0.25 percent, for the bark of the twigs, 0.12 to 0.16 percent, for the bark of the trunk, 0.08 to 0.12 percent.

## Chapter VI

### Essential Oils

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#### OIL OF ANISE

528. **Characters:** sp. gr. 0.978 to 0.988 at 25° C. (U. S. P.) (0.975 to 0.990 at 20° compared with water at 15.56° C., B. P.); optical rotation—2° to + 10° in a 100 mm. tube at 25° C.; refractive index, 1.544 to 1.560 at 20° C.; soluble in 3 volumes of 90 percent alcohol; congealing point not below 15° C. (Does not liquefy again below 17° C., B. P.)

529. **Anethol, the valuable constituent of the oil** may be determined approximately by fractional distillation, the portion of oil distilling between 225° and 235° C. being taken as approximately pure anethol. The percent is generally as high as 85 and should be not less than 80. A rough estimation of anethol may be made by chilling the oil until congelation has taken place, then pressing the crystals between folds of filter paper, maintaining a temperature below 14° C. and weighing.

#### OIL OF BERGAMOT

530. **Characters (N. F.):** Sp. gr., 0.875 to 0.880; optical rotation, — 8° to + 24°; refractive index, 1.465 to 1.466. It owes its agreeable odor largely to linalylacetate, of which it contains not less than 36 percent. The quantity may be determined by saponification (130), each mil of half-normal alkali corresponding with 0.09808 gm. of the ester.

#### OIL OF BITTER ALMOND

531. **Characters:** Sp. gr., 1.038 to 1.060 at 25° C.; optical rotation, nil or slight; not exceeding —0°10' in a 100 mm. tube at 25° C.; refractive index, 1.5428 to 1.5439 at 20° C.; dissolves to a clear solution in 2 volumes of 70 percent alcohol.

532. **Assay of the oil for benzaldehyde** may be made according to (634).

533. The U. S. P. IX gives an **assay for hydrocyanic acid** substantially as follows: Dissolve 0.75 gm. of crystallized magnesium sulphate in 40 mls of distilled water, add 2.5 mls of normal sodium hydroxide solution. Mix, add 2 drops of solution of potassium neutral chromate (10 percent), then cautiously, drop by drop, tenth-normal silver nitrate, until the solution acquires a permanent reddish tint, avoiding any excess. Pour the mixture into a 100 ml Erlenmeyer flask containing 1 ml of the sample, accurately weighed, mix well and titrate immediately and expeditiously with tenth-normal silver nitrate to the production of a red tint which does not disappear on shaking. Each ml of the volumetric silver solution corresponds to 0.0027 gm. of hydrocyanic acid. The U. S. P. IX requires that the oil shall contain not less than 2 percent nor more than 4 percent of hydrocyanic acid. [Owing to the instability of the acid, it is not easy to maintain a standard for this preparation, which may well be replaced in prescribing by diluted hydrocyanic acid. An oil (or spirit) free from hydrocyanic acid might be used for flavoring, but for this use the official benzaldehyde is to be preferred.]

### OIL OF CAJUPUT

534. **Characters:** Sp. gr., 0.912 to 0.925 at 25° C.; optical rotation, not to exceed—4° in a 100 mm. tube at 25° C.; soluble in an equal volume of 80 percent alcohol. The oil contains cineol of which the British Pharmacopoeia requires 45 percent. The U. S. P. fixes no cineol standard. For assay see (681) to (684).

### OIL OF CARAWAY

535. **Characters:** Sp. gr., 0.900 to 0.910 at 25° C.; optical rotation, between +70° and +80° in a 100 mm. tube at 25° C.; soluble in 8 parts of 80 percent alcohol.

536. **This essential oil, if genuine,** consists chiefly of a mixture of carvone ( $C_{10}H_{14}O$ ) and the

terpene *d*-limonene (citrene, carvone) which is a constituent of many essential oils, more particularly the citrus oils and those of celery, dill, fennel and erigeron. The former has a specific gravity of about 0.964, the latter of 0.850, approximately. The volume percent of carvone, on the assumption that carvone and limonene alone are present, will be found by deducting 0.85 from the specific gravity of the oil and multiplying the remainder by 877.2. The specific gravity of the oil according to U. S. P. IX is 0.900 to 0.910, which would indicate according to the foregoing calculation between 43.9 and 52.6 percent by volume of carvone, whereas the requirement is for not less than 50 percent. (The fact that the official specific gravity is taken at 25° C. instead of 15°, the standard temperature probably adopted in the calculation, will add something to the calculated percentages, but will leave them still low.) The British Pharmacopoeia gives the specific gravity of the oil as 0.910 to 0.920; the German Pharmacopoeia has 0.907 to 0.915, the latter making the percent range from 50 to 57. The available data are not sufficient to decide the question whether 0.900 (at 25° C.) is too low a figure for a minimum specific gravity or whether 50 percent of carvone is too high a requirement.

537. **The U. S. P. IX assay** is made by causing a measured portion of the oil to react with a neutral saturated solution of sodium sulphite at a water bath temperature, neutralizing the mixture from time to time with diluted acetic acid until no further reaction takes place (i. e. until no coloration appears upon addition of a few more drops of phenolphthalein indicator and heating fifteen minutes). The experiment is conducted in a cassia flask, the residual oil when cool being brought into the graduated neck of the flask where its volume is accurately measured, the loss indicating the quantity of carvone present.

538. **A promising method** of separating carvone from other constituents of an essential oil has been suggested by Kremers and Schreiner\*, depending on

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\*Pharm. Review, 14, p. 76.

conversion of the carvone into its oxime by treatment with hydroxylamine hydrochloride. Thus far the method has not been elaborated so as to yield strictly quantitative results.

539. **Another possible procedure** is to fractionate the oil, reserving the portion distilling above 200° C. as containing the whole of the carvone. A mixture of 20 parts of this fractionated oil with 5 parts of alcohol and 1 part of water of ammonia (10%) is treated with hydrogen sulphide, when the sparingly soluble carvone hydrosulphide separates. This may be collected, recrystallized from methyl alcohol and then decomposed by treatment with alcoholic solution of potassium hydroxide, and the regenerated carvone, distilled in a current of steam.

539½. The requirement of the **British Pharmacopoeia** is that when fractionally distilled at the rate of one drop per second (quantity not specified), not less than 50 percent (by volume) distils at a temperature above 200° C.

## OIL OF CHENOPODIUM

### (WORMSEED)

540. **Characters:** Sp. gr., 0.955 to 0.980 at 25° C.; optical rotation—4° to—10° in a 100 mm. tube at 25° C.; soluble in 8 volumes of 70 percent alcohol.

## OIL OF CINNAMON

### (OIL OF CASSIA)

541. **Characters:** Sp. gr., 1.045 to 1.063 at 25° C.; optical rotation, from + 1° to—1° in a 100 mm. tube at 25° C.

542. The characteristic odor and taste of essential oils distilled from cinnamon and cassia barks is due to cinnamic aldehyde, which is present generally in the proportion of at least 80 percent of the weight of the oil. Assay of the oil is made by the sulphite or bisulphite process. The official U. S. P. assay is identical with that for oil of caraway (527) the requirement being that the oil shall show not less than 80



percent (volume) of cinnamic aldehyde. Following are details of the assay by sodium bisulphite as worked out by Schimmel & Co.\* and generally adopted as a basis for commercial transactions. Place in a cassia flask by aid of a pipette 10 mls of the sample, add an equal volume of a 30 percent solution of sodium bisulphite, shake the flask and place it on a boiling water bath. After the solid mass at first formed has liquefied, add more of the bisulphite solution and continue the heating, shaking the flask from time to time. Add the bisulphite solution little by little until the flask is nearly four-fifths filled and continue the heat until the odor of cinnamon has disappeared. Finally cool the flask and add sufficient bisulphite solution to bring the lower limit of the oily layer to the zero point of the scale. Read off the volume of the oil and deduct the reading from ten to find the volume of the cinnamic aldehyde contained in the sample.

#### OIL OF CITRONELLA

543. **Characters** (unofficial): Sp. gr., of Singapore oil, 0.886 to 0.900 at 15°C.; of Ceylon oil, 0.900 to 0.920 at 15° C.; optical rotation, Singapore oil—0° 34' to—3°; Ceylon oil—5° to 21°; "geraniol" content, Singapore oil 80 to 91 percent, Ceylon oil 50 to 70 percent.

544. **The constituent of the oil** to which it owes its characteristic odor is the aldehyde, citronellal,  $C_{10}H_{18}O$ ; with this is associated the corresponding alcohol,  $C_{10}H_{20}O$  and another alcohol, related in a similar manner to citral, viz.: geraniol  $C_{10}H_{18}O$ .

545. **The assay of oil of Citronella** has in the past sought to determine its content of what was called "geraniol," in fact the total alcohol and aldehyde constituents of the oil. V. Boulez in 1912 proposed a sulphite method, justly making the aldehyde the constituent of chief consequence, although his method included also a determination of the alcohols present. Schimmel's Report (Oct. 1912) declared that of all the methods then known, that by phthalic anhydride was the only one of any real value.

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\*Bericht, Oct. 1890, 12.

**546. The method is carried out as follows:** about 2 gm. of the anhydride with 2 gm. of the sample are heated on the water bath 2 hours with 2 mls of benzene in an acetylation flask. When cool, the mixture is shaken 10 minutes with 60 mls of half-normal potassium hydroxide. During this manipulation the flask is closed with a glass stopper. At the end of this time all the aldehyde (citronellal) is converted into neutral potassium phthalate, and the acid geraniol ester into its potassium salt. The excess of alkali is now titrated back with half-normal sulphuric acid. By deducting from the quantity of alkali which corresponds with the phthalic acid used in the experiment the quantity consumed in the reaction, the equivalent in alkali of the geraniol which has been combined with the phthalic acid is obtained, and from this result the percentage of geraniol is calculated. (Equivalent of geraniol = 0.17015.)

**547. Improved acetylation method** of J. Dupont and L. Labaurie\*. The oil is first treated with phenylhydrazine hydrochloride, to convert the citronellal into its oxime which is changed by acetylation into a nitrile which resists saponification so that the final treatment with alkali gives only "geraniol," i. e. the alcohol content of the oil. The assay process in a somewhat modified form is as follows†. With 10 mls of a freshly prepared alcoholic solution of phenylhydrazine, mix 1 gm. of Ceylon or 0.5 gm. of Java (Singapore) oil in a glass stoppered 50 ml flask. Let the mixture stand undisturbed one to one and one half hours, then add 20 mls of tenth-normal hydrochloric acid, mixing the liquids by a gentle swaying of the flask, add 10 mls of benzene, shake the mixture well and transfer to a separator. After 15 minutes, draw off the acid solution (30 mls) and filter. To 20 mls of the filtrate add 10 drops of methyl orange indicator (1 : 2000) and titrate with tenth-normal potassium hydroxide to a distinct yellow color. Make a blank test omitting the oil, to

\*Bull. Roure-Bertrand fils., 1912 (5) 3.

†Schimmel's Report, Oct. 1914, 42.

ascertain the alkalimetric titre of the phenylhydrazine solution. Let (a) represent the number of mils of volumetric alkali consumed in the first titration and (b) of that consumed in the control, then the quantity of citronellal present in the sample taken (s) will be equal to  $(a-b) \times 1.5$  (since only two-thirds of the sample was represented in the titration), multiplied again by 0.0154, the quantity of citronellal corresponding with 1 mil of the volumetric alkali. The percent of citronellal will be accordingly  $(a-b) \times 1.5 \times 1.54 \div s$ .

**548. The total alcohol and aldehyde** content of the oil is to be found by acetylizing a portion of the oil without previous treatment with phenylhydrazine. The formula for percentage will be  $(a-b) \times 1.54$ . In each formula # stands for the weight in grammes of the sample taken for the assay.

#### OIL OF CLOVE

**549. Characters:** Sp. gr., 1.038 to 1.060 at 25° C.; optical rotation, not above 1° 10' in a 100 mm. tube at 25° C.; soluble in 2 volumes of 70 percent alcohol.

**550. The essential oil of clove** consists largely (70 to 85 percent) of eugenol, a phenolic body. Quantitative determination of this constituent of the oil is effected by the method outlined in (135).\* The U. S. P. requirement is for a minimum content of 82 percent by volume of eugenol; the British and some other leading pharmacopoeias make the minimum 85 percent. In the U. S. P. test, 10 mils of the oil are shaken in a cassia flask five minutes with 50 mils of solution of potassium hydroxide, and the mixture is then heated 10 minutes on a water bath. When cool, the volume of the residual oil is read off on the graduated neck of the cassia flask.

**551. More exact results** are reached by the method of R. Reich.† Heat 15 minutes on a water bath, under a reflux condenser, 1.8 gm. of the oil with

\*Umney in Pharm. Journ., III, 25, p. 950.

†Zeitschr. Nahr. Genussm., 1909, 18, 401-12.

20 mls of a 5 percent solution of sodium hydroxide. After cooling, extract the residual oil with ether. Dilute the alkaline aqueous solution to 30 mls with 5 percent sodium hydroxide solution. Of this take 15 mls and shake with 5 mls of 25 percent sulphuric acid, 6 gm. of sodium chloride and 20 mls of pentane. When the fluids have separated, transfer an aliquot portion of the pentane solution to a tared flask, evaporate off cautiously nearly the whole of the pentane, then add a few drops of isopropyl chloride, close the flask with a stopper perforated for two tubes, one to introduce a current of dry warm air, the other terminating in a platinum blowpipe jet. The air and vapor issuing from this jet is directed against a heated copper gauze. When a green color is no longer produced, the evaporation is known to be complete, and the residue of eugenol in the flask is weighed.

**552. Assay by acetylation** according to A. Verley and Fr. Bolsing\* (in absence of foreign alcohols and phenols) yields satisfactory results, and the method is commended by E. C. Spurge†, who finds it superior in accuracy to the method of Umney (540), and particularly to that of H. Thoms, which is at once tedious and inexact. The method commonly employed shows only the uncombined eugenol in the sample, and this is sufficient for pharmaceutical standardization. The oil however contains also a notable amount (7 to 17 percent) of eugenol esters. Determination may be made of these by saponification followed by acetylation by the method of Verley and Bolsing.

**553. The method of Thoms\***, alluded to above, consists in converting the eugenol into its benzoyl derivative by action of benzoyl chloride and separating and weighing this crystalline compound.

**554. The official Eugenol** of the U. S. P. is described as having a specific gravity of 1.064 to 1.07 at 25° C. and a boiling point between 250° and 255° C. No quantitative standard of purity is given, but

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\*Ber. d. pharm. Gesellschaft, 1901, 3359-62.

†Ibid., I, p. 283.

1 mil must form a clear solution with 12 mls of the official sodium hydroxide T. S., and 18 mls of water. The fact that eugenol as well as oil of cloves becomes darker and thicker with age and exposure to the air, explains why exact standards of strength are impracticable.

### OIL OF CORIANDER

555. **Characters:** Sp. gr., 0.863 to 0.875 at 25° C.; optical rotation, + 8° to + 13° in a 100 mm. tube at 25° C.; soluble in 3 volumes of 70 percent alcohol.

### OIL OF CUBEB

556. **Characters:** Sp. gr., 0.905 to 0.925 at 25° C.; optical rotation—20° to — 40° in a 100 mm. tube at 25° C.; alcoholic solution neutral to litmus.

### OIL OF EUCALYPTUS

557. **Characters:** Sp. gr., 0.905 to 0.925 at 25° C.; soluble in 4 volumes of 70 percent alcohol. When 2 mls of the oil are mixed with 4 mls of glacial acetic acid and 3 mls of a saturated solution of sodium nitrite are gradually added, no crystals of phellandrene nitrite appear on gentle stirring. Standard of U. S. P., not less than 70 percent by volume of cineol. For assay see (681) to (684).

### OIL OF FENNEL

558. **Characters:** Sp. gr., 0.953 to 0.973 at 25° C.; optical rotation, + 12 to + 24 in a 100 mm. tube at 25° C.; soluble in 8 volumes of 80 percent alcohol and in 1 volume of 90 percent alcohol, the solution neutral to litmus; congealing point, after cooling to 0° C. not below 3° C.

### OIL OF JUNIPER

559. **Characters:** Sp. gr., 0.854 to 0.879 at 25 C.; optical rotation, 0.° to—15° in a 100 mm. tube at 25 C.; soluble in 4 volumes of official alcohol, with not more than slight cloudiness.

## OIL OF LAVENDER

560. **Characters:** Sp. gr., 0.875 to 0.888 at 25° C.; optical rotation—1° to—10° in a 100 mm. tube at 25° C., soluble in 3 volumes of 70 percent alcohol.

561. **Assay of the oil** may be made for linalyl acetate by the saponification method. This is not the only constituent giving the oil its usefulness in perfumery, and the proportion present cannot be taken as a correct measure of its value. It is stated that the English oil contains 7 to 10 or 12 percent of esters calculated as linalyl acetate, while the French oils contain 30 to 40 percent or more. The assay of the U. S. P. IX establishes only a maximum limit for foreign esters, which must not consume in saponification (for 10 mils of the oil) more than 0.3 mil of half normal alcoholic potassium hydroxide. Each mil of the volumetric alkali corresponds with 0.09808 gm. of linalyl acetate.

## OIL OF LEMON

562. **Characters:** Sp. gr., 0.851 to 0.855 at 25° C.; optical rotation, + 57° to + 64° in a 100 mm. tube at 25° C.; refractive index, 1.4744 to 1.4755 at 20° C. The flavoring constituent of the oil is citral, of which the proportion is however, small. The U. S. P. IX requires not less than 4 percent of aldehydes calculated as citral. For assay methods see (674) to (680).

## OIL OF MUSTARD, VOLATILE

563. **Characters:** Sp. gr., 1.013 to 1.020 at 25° C.; optically inactive; the oil distills completely between 148° and 154° C. and both the first and the last portions of the distillate have nearly the same specific gravity as the original oil. For assay of the oil see (517) and (518).

## OIL OF MYRISTICA (NUTMEG)

564. **Characters:** Sp. gr., 0.859 to 0.924 at 25° C. (U. S. P.) [0.870 to 0.925, B. P.], optical rotation, + 12° to + 30° in a 100 mm. tube at 25° C.; soluble in 3 volumes of 90 percent alcohol.

## OIL OF ORANGE (SWEET)

565. **Characters:** Sp. gr., 0.842 to 0.846 at 25° C.; optical rotation, not less than + 94° at 25° C.; refractive index, 1.4723 to 1.4737 at 20° C.

## OIL OF PEPPERMINT

566. **Characters:** Sp. gr., 0.896 to 0.908 at 25° C.; optical rotation—23° to—33° in a 100 mm. tube at 25° C.; soluble in 4 volumes of 70 percent alcohol, showing not more than slight opalescence and no separation of oil globules.

567. **Oil of peppermint contains** not only menthol but menthyl acetate (and in small proportion menthyl isovalerate), also some menthone, a product of oxidation of menthol. In assays of the oil, the last of these is not taken into account, but determinations are made of total menthol and of menthyl esters, calculated as menthyl acetate. The latter determination is often omitted as of comparatively small importance. It should however, be made, and this determination should logically precede that of menthol.

568. **Assay for esters.** Saponify as described in (130) ten mls of the sample, accurately weighed, using 25 mls of half-normal alcoholic potassium hydroxide. Titrate the excess of alkali with half-normal sulphuric acid, subtract the number of mls of volumetric acid required for the titration from 25 multiply the remainder by 9.909 and divide the product by the weight of the sample to find the percent of esters estimated as menthyl acetate. (To find the percentage of menthyl present in the form of esters, substitute for 9.909 the constant 7.808. In either case the result is only approximate, although closely so, since other esters are present in small proportion with the menthyl acetate.)

569. **To determine total menthol,** introduce into a flask provided with a vertical tube 1 meter long to serve as a condenser, fitted by a ground-glass joint (acetylation flask) about 10 mls of the sample (accurate weighing or measuring is unnecessary).

Add 10 mls of acetic anhydride and 2 gm. of anhydrous sodium acetate. Boil the mixture gently an hour or longer; when cool transfer it to a separator and shake repeatedly with distilled water and then with a 5 percent solution of sodium carbonate (monohydrated) until the mixture shows with phenolphthalein indicator a pink tinge. Dehydrate the separated oil with anhydrous sodium sulphate (or with fused calcium chloride) and filter. Weigh (accurately) into a 100 ml flask 5 gm. of the dried acetylated oil, add 50 mls of half-normal alcoholic potassium hydroxide solution and boil one hour under a reflux condenser. After cooling, titrate the residual alkali with half-normal sulphuric acid, using phenolphthalein as indicator. According to the generally accepted formula, the percent of total menthol =

$$\frac{A \times 7.808}{B - (A \times 0.021)}$$

A representing the number of mls of standard alkali (half-normal) consumed in neutralizing the acid of acetylation, and B the weight of acetylated oil taken for saponification, which for simplifying calculations should be exactly 5 gm.

570. The basis of this percent, however, is not the original oil but the oil after saponification, containing the whole of the menthol as such and hence weighing less than it did in the natural state. We have already determined the quantity of menthol present in the form of esters. We have therefore data for the required correction. If we let E stand for the percent of menthol present as esters, the corrected percent of total menthol will be found by multiplying the percent calculated from the U. S. P. formula by the factor,

$$\frac{100 - (E \times 0.21)}{100}$$

The complete formula therefore is:

$$\text{total menthol (M)} = \frac{A \times 7.808 \times (1 - E \times 0.021)}{B - A \times 0.021}$$

The correction may be said to be unimportant, but a pharmacopoeial requirement of 50 percent which is satisfied by an article containing only 49.5 percent shows a laxity in the use of language that ought not to be tolerated in legal standards of strength or purity.



571. **An alternative formula** which is sufficiently exact for the purposes of a pharmacopoeial standard is the following: Percentage of total menthol =

$$\frac{A \times 7.808}{B - [A - (C \div 2.5)] \times 0.021}$$
 in which C stands for the number of mls of half-normal potassium hydroxide consumed in saponifying 10 gm. of the oil. The expression  $C \div 2.5$  is correct in case the oil contains 50 percent of total menthol. If the quantity is smaller than this, the factor 2.5 is inexact, although in no case will it be less than 2. It will be approximately correct if we take it as  $2 +$  a fraction equal to the percent of menthol present. However, if the formula is employed only for determining whether or not a sample of oil is up to the pharmacopoeial standard, the empirical formula as it stands may be used with confidence.

#### OIL OF PIMENTA

572. **Characters:** Sp. gr., 1.018 to 1.048 at 25° C.; optical rotation, 0° to -4° in a 100 mm. tube at 25° C.; soluble in an equal volume of 90 percent alcohol, also in 2 volumes of 70 percent alcohol. The important constituent of this oil is eugenol. The U. S. P. IX requires that the percent by volume of this constituent shall not be less than 65, the assay being made as in the case of clove (540).

#### OIL OF PINE NEEDLES

573. **Characters:** Sp. gr., 0.853 to 0.869 at 25° C.; no portion of the oil distills below 170° C.

#### OIL OF ROSEMARY

574. **Characters:** Sp. gr., 0.894 to 0.912 at 25° C., U. S. P.; (0.892 to 0.920, B. P.); optical rotation, -2° to + 15°, B. P.; refractive index, 1.463 to 1.473 at 25° C., B. P.; soluble in 10 volumes of 80 percent alcohol, U. S. P. Requirements, a content of not less than 2.5 percent of esters, estimated as bornyl acetate and not less than 10 percent of total borneol, U. S. P. IX. The British Pharmacopoeia requires not less than

1.8 percent of esters, calculated as bornyl acetate, and not less than 10 percent total alcohols calculated as borneol. According to Allen's Commercial Organic Analysis the percentages of these constituents in genuine rosemary oil are resp. 5 to 6 for esters and 17 to 18 for alcohols.

575. **The assay is made** just as in the case of oil of peppermint (558) and (559), using the factor 9.808 for calculating the ester, and for percent of borneol the

expression  $\frac{A \times 7.707}{B - (A \times 0.021)}$ , the symbol A standing

for the number of mls of half-normal alcoholic potassium hydroxide required to neutralize the acetylated oil, and B for the weight (10 gm.) of acetylated oil taken. The result is the percentage of saponified oil, not of the original oil which weighs a little more. The formula should be corrected just as in the case of oil of peppermint (560).

#### OIL OF SANTAL

576. **Characters:** Sp. gr., 0.965 to 0.980 at 25° C., U. S. P.; optical rotation, -15° to -20° in a 100 mm. tube at 25° C., U. S. P.; refractive index, 1.498 to 1.508 at 25° C., B. P.; soluble in 5 volumes of 70 percent alcohol; minimum content of alcohols calculated as santalol, 90 percent.

577. **The assay is made** according to the method described in (558-559). The percentage of santalol

is equal to  $\frac{A \times 11.11}{B - (A \times 0.021)}$ , the values of A and B

being those given in (575). There is some uncertainty about the molecular formula for santalol, which is taken by the U. S. P. as  $C_{15}H_{26}O$ , and the foregoing formula for determining it is based on this assumption. Even if it should turn out that the molecular formula is inaccurate, its provisional acceptance gives us a basis for standardizing the oil.

#### OIL OF SASSAFRAS

578. **Characters:** Sp. Gr., 1.065 to 1.077 at 25° C.; optical rotation, from +3° to +4° in a 100 mm.

tube at 25° C.; soluble in 2 volumes of 90 percent alcohol, forming a solution neutral to litmus. The oil contains as its most important constituent safrol, having the empirical formula  $C_{10}H_{10}O_2$ , found also in oil of camphor. The safrol may be removed quantitatively by freezing.

#### OIL OF SPEARMINT

579. **Characters:** Sp. gr., 0.917 to 0.934 at 25° C. (U. S. P.); 0.925 to 0.940 (B. P.); optical rotation, —38° to —55° in a 100 mm. tube at 25° C.; soluble in 1 volume of 80 percent alcohol, generally becoming turbid on further dilution. The oil contains as its valuable constituent carvone; U. S. P. standard, not less than 43 percent by volume, determined as in the case of oil of caraway (536).

#### OIL OF TURPENTINE, RECTIFIED

580. **Characters:** Sp. gr., 0.856 to 0.865 at 25° C.; optical rotation, variable; soluble in 5 volumes of alcohol; 90 percent of the oil distils between 154° and 170° C.

#### OIL OF THYME

581. **Characters:** Sp. gr., 0.894 to 0.930 at 25° C.; soluble in 2 volumes of 80 percent alcohol. The oil is slightly laevorotatory, but the dark color of the oil often makes polariscope readings difficult. The oil contains thymol and other phenols which furnish a basis for standardization. The assay is made as in the case of oil of clove, by noting the diminution in volume under saponification. See (135) and (550).

## Chapter VII

### Epinephrine and Glandular Extracts

#### EPINEPHRINE (ADRENALINE)

582. **There has not as yet been devised** any method of extracting epinephrine quantitatively from dried suprarenals or other similar preparations used in medicine. The best we can do is to apply biological tests, such as that described in Part II of the U. S. P. IX (page 608). Several colorimetric methods of assay have been described which are of some practical value, although none of them are absolutely conclusive inasmuch as the color reactions on which they depend are given by some other related bases.

583. **Colorimetric assay of A. Seidell,\*** for testing dried suprarenals (adopted in U. S. P. IX). Mix 0.01 gm. of the sample with 10 mls of water and 0.005 gm. of manganese dioxide, macerate one hour, filter and compare the color developed with standards prepared from cobaltous chloride and gold chloride. The color produced if the sample contains 0.2 percent of epinephrine is matched by a solution containing in 10 mls, 1.85 mls of the official cobaltous chloride test solution and 0.95 mls of the diluted gold chloride test solution. The proportions to match the color produced by a sample containing 0.4 percent of epinephrine are, in 10 mls, 2.95 mls of cobaltous chloride solution and 1.25 mls of diluted gold chloride solution; for 0.6 percent epinephrine, 4.05 mls of cobaltous chloride solution and 1.35 mls of diluted gold chloride solution; for 0.8 percent epinephrine, 5.15 mls of cobaltous chloride solution and 1.55 mls of diluted gold chloride solution. The color standards may be kept indefinitely without change in hermetically sealed glass tubes.

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\*Journ. Biolog. Chem., 1913, 197; compare an older method, by Seidell and Hare in Am. Journ. Pharm. 1911, 557.

**584. Alternative method** of O. Folin, W. B. Cannon and W. Denis.\* A special reagent is employed, prepared by boiling gently for two hours 100 gm. of sodium tungstate and 80 mls of 85 percent phosphoric acid with 750 mls of water and making up when cold to 1 liter. Digest 1 gm. of the sample with 1 mil of diluted hydrochloric acid (10 percent) and 25 mls of water 1 hour, then heat gradually to boiling, add 0.25 gm. sodium acetate, boil 2 minutes, add water 20 mls, when cold make up with water to 51 mls. By filtration, preferably with aid of a centrifuge, obtain a clear solution of which 5 mls represents 0.1 gm. of the sample. Transfer to a 100 mil measuring flask 5 mls of this solution, add 2 mls of the foregoing reagent and 20 mls of a saturated solution of sodium carbonate, shake and after 10 minutes make up with water to 100 mls. Into a second 100 mil measuring flask put 1 mil of a freshly prepared solution of uric acid (1 : 1000), adding to this also 2 mls of the reagent and 20 mls of sodium carbonate solution, and after 10 minutes filling with water to the mark. By colorimetric comparison, deduce the quantity of epinephrine present, remembering that the coloring power of epinephrine is just three times that of uric acid.

**585. Dr. Torald Sollmann** has recently proposed\* a simple method of testing the activity of anesthetic hypodermatic tablets of epinephrine by the effect they produce when injected intracutaneously into the human forearm. The solution injected must be very dilute, 1 mil containing about 0.00125 mg. of epinephrine (i. e. 1 : 800,000). The quantity of such solution injected is only 0.2 to 0.4 mil. The dilutions used for the tests are to be made with a recently boiled 1 percent solution of sodium chloride. Comparison is made with the effect produced by a solution of known strength, the quantity of epinephrine being indicated by the extent, intensity and duration of the blanching of the surface. A number

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\*Journ. Biolog. Chem., 1913, 472.

\*Journ. Am. Pharm. Assoc., 1918, 435.

of tests are made, so that the comparative effects of the known and unknown solutions can be finally judged with a fair degree of exactness. The method, however, is not likely to be practised outside of the physician's office.

### PITUITARY EXTRACT

586. **We have as yet no chemical tests** by which to standardize preparations made from the pituitary gland. The subject would not be mentioned here but for the fact that the U. S. P. IX has introduced for Liquor Hypophysis a biological test, as follows: One mil of the solution, diluted 20,000 times, has the same activity on the isolated uterus of the virgin guinea-pig as a 1 to 20,000,000 solution of beta-iminazolyethylamine hydrochloride when tested as directed by the U. S. Hygienic Laboratory. Exception is taken to this test by P. S. Pittenger and C. G. Vanderkleed on the grounds (1) that the substance chosen as a standard is not uniform in its activity—a fatal objection: (2) that the substance chosen as a standard is nearly 3 times as toxic (in doses having equal action on the uterus) as the solution of hypophysis: (3) that the product which has heretofore been employed medicinally is 3 to 5 times as active (judged by the official test) as the pharmacopœial standardized product. Inasmuch as no standard is fixed for desiccated hypophysis, it would seem wise to drop the official requirement for solution of hypophysis, leaving the question of standardization with manufacturers as heretofore.

## THYROIDES, DRIED

**587. The medicinal effects of dried thyroids** or of thyroid extracts have been commonly attributed to the iodine compound discovered by Baumann in 1906 and named by him iodothyrene. It is true that it has not been proved beyond question that this is the sole active constituent of the glands, yet it furnishes a plausible basis for a standardization of thyroid preparations for medicinal use, and as such has been adopted by the U. S. P. IX. Determination of organic iodine in such preparations is made, according to L. W. Riggs\*, by fusing the dry substance in a nickel crucible with twice its weight of sodium hydroxide for some time at a barely perceptible red heat, adding then one fourth more sodium hydroxide, followed by sodium nitrate added little by little. The fused mass is cooled, extracted with water and the solution filtered, the crucible and filter washed repeatedly with hot water, and the filtrate and washings finally made up to 100 mls. An aliquot part of the solution is transferred to a separator with 10 mls of carbon tetrachloride and cautiously acidified with 25 percent sulphuric acid. The liberated iodine goes into solution in the carbon tetrachloride, the quantity being estimated colorimetrically by comparison with a solution of iodine of known strength. In this form the assay process is certainly far from satisfactory.

**588. The official assay process.** This differs essentially from the Riggs process in that the iodine is brought into the form of iodate which is then made to react with potassium iodide in acid solution, setting free the iodine, which is determined by titration with thiosulphate solution. The following is the procedure: Prepare a fusion mixture composed of 8.65 gm. of anhydrous potassium carbonate, 6.65 gm. of anhydrous sodium carbonate and 4.70 gm. of potassium nitrate. Mix 1 gm. of the sample intimately with 15 gm. of the fusion mixture in a nickel crucible and

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\*Journ. Am. Chem. Soc., 1909, 710-717.

spread evenly over the surface the remainder of the fusion mixture. Ignite at a red heat until the carbon is burned off, cool the crucible and dissolve the "melt" in 175 mls of water with aid of heat. Add to the solution 50 mls of a freshly prepared solution of chlorinated soda (2.4 percent Cl) add phosphoric acid gradually until the appearance of a yellow color (chlorine) indicates a slight excess, then add further 5 mls of phosphoric acid and boil the mixture half an hour. When cold, add 10 mls of a 1 percent solution of potassium iodide and titrate the liberated iodine with freshly prepared two-hundredth-normal sodium thiosulphate, using starch solution as indicator. Each mil of the standard thiosulphate solution corresponds with 0.1058 mg. (erroneously stated in the U. S. P. as 0.01058 mg.) of iodine in the form of combination peculiar to the thyroid.

589. The assay is convincing only if it is certain that the sample examined (dry thyroids) contains no foreign substance. The text of the Pharmacopoeia states that "dried thyroids must be free from iodine in inorganic or any other form of combination than that peculiar to the thyroid," but gives no tests to establish the absence of the interdicted compounds.

590. **The U. S. P. standard** seems to have been fixed quite arbitrarily. Since the proportion of iodine present in different samples of dried thyroids ranges from 0.08 to 0.50 percent as reported by competent analysts, it would seem to be a difficult task for the manufacturer to supply a product containing "not less than 0.17 nor more than 0.23 percent." The U. S. P. permits the use of the thyroid glands from (all?) animals used for food by man." Practically it has been the glands from the sheep that have been especially made the subject of study. N. H. Martin\* found in these, from sheep raised in England, between 0.3 and 0.4 percent of iodine, in averages including from 200 to 1000 lobes, taken in every month in the year, the season apparently making no important difference.

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\*Yearbook of Pharmacy, 1912, 408-10; 1913, 487-8.



## Chapter VIII

### Organic Principles and Synthetics

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#### ACETANILID

591. **Assay by precipitation as aniline tri-bromide** according to A. Seidell. The acetanilide is decomposed by heating with hydrochloric acid, a standard bromine solution is added as long as a precipitate of aniline tri-bromide is formed, each mil of the standard solution corresponding with 0.01 gm. of acetanilide. The procedure is as follows: Place in a 200 mil Erlenmeyer flask about 0.5 gm., accurately weighed, of the sample; add 60 mls of diluted hydrochloric acid (1 part of the strong acid in five, by weight) and heat on a steam bath one hour (or boil energetically five minutes). Titrate the hot solution with a standard bromine solution which is to be added until just in excess as shown by the appearance of a yellow tint in the solution. The bromine solution is prepared by dissolving 10 gm. of potassium hydroxide in 10 mls of distilled water, cooling the solution to 15°C. and adding bromine as long as it is dissolved, diluting the solution somewhat, boiling to expel excess of bromine, and making up with distilled water to a volume of 200 mls. This solution is then standardized against pure acetanilid so that one mil corresponds with 0.01 gm. of acetanilid.

592. **To standardize the bromine solution,** weigh accurately about 25 mg. of pure acetanilide, previously dried 24 hours in a desiccator over sulphuric acid. Dissolve it in 2 mls of official hydrochloric acid diluted with an equal volume of water, and boil the solution 5 minutes. Add 10 mls of hot water and titrate the hot solution with the bromine solution to appearance of a yellow tint. Let a represent the number of mg. taken, b the number of mls

of the bromine solution consumed in the titration and  $v$  the volume of the bromine solution. Add water sufficient to bring the volume to  $v \times (a \div b)$ . Example: 25 mg. of pure acetanilid consumes 16 mls of the bromine solution in the titration. If the volume of the solution ( $v$ ) is 185 mls, add water sufficient to make  $185 \times (25 \div 16) = 289 +$  mls.

**593. Method of Turner and Vanderkleed.\*** The acetanilid is saponified by heating with sodium hydroxide; the solution, freed from aniline by washing with ether, is acidified with phosphoric acid, and the acetic acid resulting from the splitting up of the acetanilid is distilled off and determined by titration with volumetric potassium hydroxide. In detail, weigh accurately 0.5 gm. of acetanilide, previously dried at  $100^{\circ}$  C., place this in a flask fitted with a reflux condenser, add 10 mls of alcohol, 5 mls of water and 1.5 gm. of sodium hydroxide, and heat on a water bath  $1\frac{1}{2}$  to 2 hours. Evaporate off the alcohol, transfer the solution to a separator, shake with 25 mls of ether in two portions, wash the ether twice with water (10 mls) to recover traces of sodium acetate, mix the aqueous washings with the solution in the separator, add 12.5 mls of phosphoric acid (85 percent) and transfer the mixture to a suitable distilling flask, taking care to rinse the separator and add the rinsings to the distilling flask. Distil with aid of a current of steam until the distillate comes over free from acid and titrate the acetic acid which resulted from the decomposition of the acetanilid with volumetric solution of potassium or sodium hydroxide. Each mil of normal alkali corresponds with 0.13508 gm. of acetanilid. Note that acetphenetidin may be determined by the same procedure, each mil of normal alkali corresponding with 0.17911 gm. of that compound. (See 604).

**594. In case both acetanilid and acetphenetidin** are present, determination of the two is possible by first ascertaining the total quantity of acetic acid yielded by the mixture and then making a determina-

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\*Amer. Journ. Pharm., April 1907, 151-6.

tion by an independent method of the acetphenetidin present, calculating the quantity of acetic acid which this would yield, deducting this from the total acid found and calculating from the remainder the quantity of acetanilid. W. O. Emory has shown\* that in mixtures of these two compounds containing not more than 75 percent of acetanilid, determination of the acetphenetidin may be made substantially by the method of (604).

594½. Mixtures containing caffeine and antipyrine, as well as acetanilide and acetphenetidin must be treated in the following manner (Emery, *ibid*). Digest the solution on a steam bath with diluted sulphuric acid to convert acetanilide and acetphenetidin respectively to aniline and phenetidine sulphates. Shake out caffeine and antipyrin with chloroform. The acetphenetidin and acetanilide may then be regenerated, and the respective quantities determined as below. Determination of antipyrine and caffeine can be made by (626).

595. The following modified procedure may be adopted. Place in a 50 mil Erlenmeyer flask about 0.15 gm., accurately weighed, of the mixture containing acetanilid and acetphenetidin, add 0.05 gm., also accurately weighed of acetphenetidin (unless it is known that the mixture contains not more than 75 per cent of acetanilid; in such case use simply 0.2 gm. of the mixture, without addition of acetphenetidin); add 2 mls of glacial acetic acid and heat gently until complete solution is effected, then add 40 mls of distilled water, previously heated to 70° C., transfer the mixture to a glass stoppered 100 mil measuring flask, containing exactly 25 mls of a fifth-normal iodine solution, rinsing the Erlenmeyer with two portions (10 mls each) of water at 40° C. and adding the rinsings to the measuring flask. Stopper the flask and mix its contents thoroughly by rotating, add 3 mls of concentrated phosphoric acid (85 percent) stopper the flask and rotate once more until copious crystallization of the acetphenetidin

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\*Journ. Ind. and Eng. Chem., 1915, 665-8.

periodide has taken place. The presence of acetanilid retards the crystallization, but if the proportion of acetanilid to acetphenetidin is not greater than three to one, continuation of the rotation, maintaining the temperature at  $48^{\circ}$  by immersion of the flask in water at that temperature, will presently bring about the desired result.

596. When cooled to room temperature fill the flask nearly to the mark with water, mix the contents thoroughly by rotation and let stand over night; then fill to the mark with water, mix thoroughly, set by for 30 minutes, filter an aliquot of 50 mils (after rejecting the first 15 mils which pass the filter) and titrate the excess of iodine with tenth-normal sodium thiosulphate. Subtract the number of mils of thiosulphate solution consumed from 25 and multiply the remainder by 0.017911 to find the quantity in grammes of acetphenetidin in the sample taken. Deduct from this, of course, the quantity, if any, of acetphenetidin added. See (606).

597. **Example.** To 0.15 gm. of a mixture of acetanilid and acetphenetidin, 0.05 gm. of acetphenetidin has been added. The mixture is found by the foregoing procedure to contain 0.085 gm. of acetphenetidin. An identical mixture yields by (593) acetic acid corresponding with 13.26 mils of decinormal alkali. Of this, the acid derived from acetphenetidin accounts for 4.75 mils, leaving 8.51 mils as the quantity furnished by the acetanilid, which therefore =  $8.514 \times 0.013508 = 0.11496$ , or 76.64 percent of the original mixture.

598. **The acetanilid** can also be directly determined in an aliquot portion of the filtrate of (596) from which the phenacetin has been precipitated. Transfer to a separator 25 mils of this filtrate, decolorize with sodium sulphite in substance, added little by little, add sodium bicarbonate in slight excess, then one or two drops of acetic anhydride, and shake out the acetanilid with three portions (50 mils each) of chloroform. Filter the chloroform through a dry filter into a 200 mil Erlenmeyer and distil by a gentle heat down to about 20 mils. Add

10 mls of water and 1 mil of concentrated sulphuric acid, digest on a steam bath until reduced to about 15 mls, add 20 mls of water and continue digestion one hour, finally add again 20 mls of water and 20 mls of concentrated hydrochloric acid and titrate very slowly, drop by drop with a standard solution of bromine, one mil of which is equivalent to 10 mg. of acetanilid until a faint yellow coloration persists. While adding the standard solution, rotate the flask to facilitate separation of the precipitate of tribrom-aniline. See (591).

599. When mixtures are to be assayed for acetanilid, they are to be brought into aqueous solution. Alcohol, if present, is to be removed by evaporation. The acetanilid is to be extracted by shaking out with several portions of chloroform under the usual precautions. Headache remedies are liable to contain besides acetanilid, which is generally present, one or more of the following, acetphenetidin, antipyrin, caffeine, aspirin or salol, the separation of which calls for exercise of a good deal of ingenuity.

## ACETIC ANHYDRIDE

600. When acetic anhydride (acetyl oxide) is dissolved in water, each molecule of it combines with one molecule of water, forming two molecules of acetic acid (acetyl hydroxide). Hence we may regard the anhydride as potentially a dibasic acid, so that its titration equivalent will correspond with one half its molecular weight (102.05), i. e. one mil of decinormal alkali will neutralize the acid produced by hydration of 0.051025 gm. of the anhydride. On this is based the very simple assay procedure of the U. S. P. IX (p. 522) which consists in dissolving a weighed quantity of the anhydride in water and titrating it with a volumetric solution of potassium hydroxide.

The method is faulty in that it does not guard against presence in the anhydride of acetic acid, which is as objectionable as any other impurity in the reagent.

601. Dr. C. L. Alsberg, Chief of the Bureau of Chemistry in Washington, proposes the following plan which is said to give reliable results. Weigh accurately about 0.15 gm. of the sample, add 10 mls of anhydrous ether and a slight excess of aniline, stopper the flask with a chloride of calcium tube and let stand one hour at room temperature. Then add a slight excess of diluted sulphuric acid and shake out the acetanilid formed with three successive portions of chloroform. Evaporate the united chloroform solutions nearly to dryness on a water or steam bath and dry the residue in vacuo over sulphuric acid. Multiply the weight of the acetanilide by 0.7555 to find the weight of acetic anhydride in the sample.

## ACETONE

602. **Acetone is converted quantitatively** into bromoform by bromine. The bromoform may then be decomposed by an alkali hydroxide, and the bromine determined in the usual manner. The method as suggested by S. M. Auld\* is as follows: One mil of the sample (or an equivalent quantity of a mixture containing acetone) is weighed accurately and made up with distilled water to a volume of 250 mls. Five mls of the mixture (which must be free from chloroform or bromoform) is placed in a 500 mil distilling flask, which is fitted with a reflux condenser and a dropping funnel. Add slowly through the funnel tube a solution containing in one liter 200 gm. of pure bromine (free from bromoform) and 250 gm. of potassium bromide, until the solution acquires a permanent faint yellow color. Heat on a water bath at 70° C. half an hour. If an excess of bromine has been added, it may be removed by warming the solution with a few drops of solution of potassium hydroxide. The mixture is distilled until no more bromoform comes over, and the distillate, together with washings from the condenser, are mixed with 50 mls of alcohol, and sufficient solid potassium hydroxide (free from chlorides) to make approximately a

ten percent solution. Heat the mixture under an efficient reflux condenser until the bromoform is completely decomposed, which requires about 45 minutes; cool the solution, neutralize accurately with dilute nitric acid and determine bromine by titration with tenth-normal silver nitrate, using potassium chromate as indicator. Each mil of the volumetric silver solution corresponds with 0.001935 gm. of acetone.

**603. Assay process of U. S. P. IX\*.** Partially fill a stoppered weighing bottle with distilled water and ascertain its exact tare. Introduce 1 mil of the acetone and weigh accurately. Dilute with distilled water to exactly one liter. Place in a 250 mil glass-stoppered flask 25 mls of normal potassium hydroxide, add exactly 25 mls of the acetone solution, then, with constant agitation of the flask, add 35 mls of tenth-normal iodine solution, and let stand 15 minutes†. Add next 26 mls of normal hydrochloric acid, and immediately titrate residual iodine with tenth-normal sodium thiosulphate, adding starch as indicator at the last. Conduct a blank test with the same quantities of the reagents used, and subtract the quantity of iodine solution consumed from that consumed in the assay. Each mil of the difference corresponds to 0.0009675 gm. of absolute acetone.

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\*The method is essentially that of Messinger, Ber., 29, 3336 (1888). See paper by L. F. Goodwin in Journ. Am. Chem., Soc. 1920, pp. 39-45.

†In cold weather, 20 minutes.

## ACETPHENETIDIN

604. **Method of W. O. Emery.\*** The determination is based on the fact that when to an aqueous solution of phenacetin there is added a solution of iodine and potassium iodide containing a mineral acid, the phenacetin is thrown down in the form of a precipitate which shortly crystallizes and is practically insoluble in an aqueous solution containing free iodine. The composition of the precipitate is expressed in the formula  $(C^2H^5O, C^6H^4NH, CO CH^3) 2 HI I^4$ . The phenacetin may be recovered from the crystalline precipitate and determined gravimetrically or else the precipitate may be separated by filtration and the excess of iodine determined volumetrically, the total quantity originally taken being known.

605. The procedure in detail is as follows: Place in a 50 mil Erlenmeyer flask 0.2 gm. of phenacetin, add 1 mil of glacial acetic acid and heat gently until solution is effected, then add 40 mls of distilled water previously heated to  $70^{\circ} C$ . Transfer the mixture to a glass stoppered 100 mil measuring flask containing exactly 25 mls of a fifth-normal iodine solution, rinsing the flask with two portions (10 mls each) of water at  $40^{\circ} C$ . and adding the rinsings to the measuring flask. Stopper the flask and mix its contents thoroughly by rotation, add 3 mls of concentrated hydrochloric acid, stopper and rotate once more until an abundant deposit of crystals has been thrown down. When the fluid has cooled to room temperature (approximately) add water to within 1 mil of the mark and set the flask aside over night, then fill with water exactly to the mark, mix thoroughly and set by for 30 minutes.

606. For the volumetric determination, filter off through a small dry filter, after rejecting the first 15 mls of filtrate, an aliquot of 50 mls, representing 0.1 gm. of the sample, and titrate this with tenth-normal sodium thiosulphate solution. Subtract the number of

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\*Journ. Ind. and Eng. Chem., Aug. 1914, 665-8.



mils of the thiosulphate solution consumed from 25 and multiply the remainder by 0.017911 for the quantity of phenacetin contained in the sample. To make the determination gravimetrically, collect the crystalline precipitate on a filter, wash it with 15 mils of the volumetric iodine solution, added in several successive portions (preferably employing suction to hasten the filtration), transfer the filter with its contents to a separator together with any remaining crystals adhering to the interior of the flask (by aid of distilled water, 15, 10 and 10 mils), add sodium sulphite in substance, little by little, until all "free" iodine is consumed, and finally shake out the fluid with three successive portions (50 mils) of chloroform, each portion being washed in a second separator with 5 mils of water. Pass the chloroform solutions through a dry filter, into a tared beaker, evaporate, dry to constant weight at 100° C. and weigh as acetphenetidin.

**607. Method of Turner and Vanderkleed.\*** The method is the same in every particular as that for determination of acetanilide given in (593), except that the factor expressing the value of the normal alkali, which for acetanilide is 0.13508, is for phenacetin 0.17911, i. e., one mil of normal alkali corresponds to 0.17911 gm. of acetphenetidin.

**608. Determination of Acetphenetidin in mixture with Salol** (Emery, Spencer and Lefebvre).† From powder, pills or tablets, the salol and acetphenetidin are extracted by repeated treatment with chloroform, the solution being evaporated to dryness in a tared beaker at a temperature not exceeding 60° C. with aid of a current of air. The residue is to be dried finally by exposure to the air for 24 hours at the room temperature.

**609. Method I.** Conversion of the acetphenetidin to phenetidine sulphate, separation of residual salol by shaking out with chloroform, regeneration of acetphenetidin, which is then extracted and determined

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\*Amer. Journ. Pharm., April 1917, 151-6.

†Journ. Ind. and Eng. Chem., July 1915, 681.

by direct weighing. Dissolve 0.2 to 0.3 gm., accurately weighed, of the dried mixture in a little chloroform and transfer to a 50 mil Erlenmeyer, drive off the chloroform by an air blast aided by gentle heat, add 10 mls of dilute sulphuric acid (1 : 10) and digest at full-steam bath heat until the liquid is reduced one half. (In this digestion some of the salol originally present is lost.) Add 10 mls of water and digest as before, then add 10 mls more of water and evaporate to 5 mls. Transfer to a small separator, using about 20 mls of water in several portions for washing the flask, and shake out residual salol with 15, 10 and 10 mls of chloroform, washing each portion in a second separator with 5 mls of water, which is finally added to the first separator. Sodium bicarbonate is then added to this aqueous solution until an excess remains undissolved, then 25 mls of chloroform and for every decigram of acetphenetidin believed to be present, 5 drops of acetic anhydride. Shake vigorously for some time, pass the chloroform into a second separator containing 5 mls of water, shake, separate and transfer the separated chloroform to a 200 mil Erlenmeyer, after filtering through a dry filter. Distil 20 mls, which is to be returned to the first separator, together with 5 mls of fresh chloroform. Repeat the extraction just as before, distilling off this time 25 mls of the chloroform, with which a third extraction is carried out exactly as before, distilling now until about 5 mls of chloroform solution remain in the Erlenmeyer. Transfer this to a tared 50 mil beaker, evaporate to apparent dryness, remove any pronounced excess of acetic anhydride by adding repeatedly one mil portions of chloroform, containing one drop of alcohol, and evaporating. Allow the residue of regenerated acetphenetidin to remain some time in a vacuum desiccator over lime—or else exposed to the air 24 hours—weighing at intervals until constant.

610. **Method II** (for determination of both acetphenetidin and salol). The salol is saponified with sodium hydroxide and the unchanged acetphenetidin is shaken out with chloroform. The salol is determined by titration with standard bromine volumetric

solution (611). Dissolve in a little chloroform a weighed quantity of the mixture estimated to contain one decigram of salol. Evaporate off the chloroform by an air current, without heat, add 10 mls of a 2.5 percent aqueous solution of sodium hydroxide, and heat for five minutes on a boiling water bath. Cool the flask quickly in running water to room temperature to prevent any considerable hydrolysis of the acetphenetidin. Transfer the liquid to a separator with a minimum quantity of water, finally rinsing the flask with 20 mls of chloroform, to be used for shaking out the acetphenetidin from the aqueous solution. Continue the extraction with 20 and 20 mls of fresh chloroform, washing this in each case in a second separator with 5 mls of water, and filtering through a dry filter into a distilling flask. Distil to a residue of about 5 mls, transfer this to a tared beaker or dish, evaporate off the remainder of the solvent by aid of a current of air, reduce to constant weight and weigh as acetphenetidin.

611. To determine the salol, transfer the aqueous solutions from the separators to a 500 ml glass-stoppered bottle, dilute with water to about 200 mls, run in from a burette 45 mls of seventh-normal bromine solution, follow with 10 mls of strong hydrochloric acid, close the flask and shake continuously one minute then at intervals during  $\frac{1}{2}$  hour. Add 10 mls of a 15 percent solution of potassium iodide and shake the closed bottle at intervals during 15 minutes. Titrate the liberated iodine with seventh-normal sodium thiosulphate solution. Subtract the number of mls of thiosulphate solution from 45. The remainder multiplied by 0.002548 gives the quantity in grammes of salol present.

## DETERMINATION OF ORGANIC ACIDS IN SALTS OF ALKALIES

612. **The general procedure** prescribed in the U. S. P. IX is to carbonize a weighed portion of the salt (about 2 gm.) in a platinum or porcelain crucible by a heat not above dull redness, treat the residue with hot water, add 50 mls of half-normal sulphuric acid boil half an hour (to expel all carbon dioxide), then filter the solution and wash the residue with hot distilled water until the washings no longer redden litmus paper, and finally to titrate the residual acid in the solution and washings with half-normal potassium hydroxide. From the result the purity percentage of the salt is calculated on the assumption that it contains only the acid radicle implied in the name of the salt. Of course in individual cases tests are provided by which the impurities most likely to be present are excluded, but the unconfirmed conclusions drawn from such an assay can never be wholly convincing, although carrying the weight of a high degree of probability if they indicate approximate purity in the salt.

613. **The procedure may be advantageously modified** in some particulars. It is a good plan, for instance, to carbonize the salt only partially, igniting perhaps for half an hour at a temperature not exceeding that of incipient redness, cool the crucible and dissolve the residue as completely as possible in hot water, filter through an ashless filter, wash the residue well with hot water, adding the washings to the filtrate, transfer the wet moist filter and contents to the crucible in which the first ignition was made, dry and ignite it at a dull red heat, when the carbon will be very quickly burned off. The slight residue remaining is to be dissolved in hot water and added to the main solution, to which meanwhile 50 mls of half-normal sulphuric acid have been added; the solution is to be boiled and titrated just as above described.

614. In case the organic acid is **one easily removable** from aqueous solution **by an immiscible solvent** (e. g. benzoic acid) the sample may be advantageously dissolved in a small quantity of water, a moderate excess of diluted sulphuric acid added and the organic acid separated by the shaking out process, taking care to wash the ethereal solution with two small portions of water, which are to be added to the residual aqueous solution. This, on evaporation and ignition to complete expulsion of sulphuric acid (615) will yield a residuum of alkaline sulphate from the weight of which it is easy to deduce that of the organic salt. The solution of the acid on the other hand can be evaporated, brought into aqueous solution and titrated. If the salt is pure, the two determinations will agree. Of the two, the latter is the more likely to be correct. See (639).

615. **Assay by conversion into a sulphate** as suggested by Elias Elvove\*. The weighed sample (approximately 0.5 gm.) is dissolved in a minimum quantity of hot water in a platinum dish, normal sulphuric acid, 50 percent more than is theoretically required, is added and the dish, loosely covered with a piece of platinum foil, is placed in a drying oven, the temperature of which is gradually increased from 100° to 150°. When the residue is completely dry, ignite at a temperature reaching full redness continued for 10 to 15 minutes. When cool add a few drops of water of ammonia, evaporate to dryness and ignite once more 10 minutes at full red heat. Cool in a desiccator and weigh. From the molecular weights respectively of the sulphate and of the organic salt in question calculate the quantity of the latter present in the sample assayed. See (617). (To make the results wholly trustworthy, add to the residue after the first weighing a few drops of normal sulphuric acid followed by excess of water of ammonia, dry and ignite 10 minutes at a red heat.)

616. **A rapid alternative plan is the following†:** Weigh the sample (approxmately 0.5 gm.) in

\*Amer. Journ. Pharm., July 1912, 289-98.

†A. B. Lyons, Journ. Am. Pharm. Assoc., 1918, 603.

a small beaker, add 10 drops of strong sulphuric acid and 20 mls of alcohol, stir well and let stand a few minutes, then decant the clear solution into a platinum or quartz crucible, wash the residue with two portions (about 3 mls each) of alcohol and add this to the crucible. Set fire to the alcohol and when it has nearly all burned off, dry the residue, consisting mostly of the organic acid together with a little sulphuric acid, and ignite it at a temperature not above dull redness. The ignition requires very little time. Cool the crucible and transfer to it the residue in the beaker, by aid of several portions of hot water. Evaporate the solution consisting almost wholly of alkali sulphate, and when quite dry, ignite it as in the foregoing paragraph. There will be practically no reduction of sulphate. (If there is reason to fear such reduction, dissolve the residue in water, add a few drops of normal sulphuric acid, followed by slight excess of ammonia, dry and ignite once more). The ignited residue consists of anhydrous alkali sulphate or sulphates, as in the foregoing process.

**617. Factors for converting anhydrous sulphate into organic salt.**

Lithium Citrate, cryst., $\text{Li}_3 \text{C}_6 \text{H}_5 \text{O}_7 + 4\text{H}_2\text{O}$ . . .	1.7094
Lithium Salicylate, $\text{Li C}_7 \text{H}_5 \text{O}_3$ . . . . .	2.6190
Lithium Benzoate, $\text{Li C}_7 \text{H}_5 \text{O}_2$ . . . . .	2.3283
Potassium Acetate, $\text{K C}_2 \text{H}_3 \text{O}_2$ . . . . .	1.1261
Potassium Benzoate, cryst., $\text{K C}_7 \text{H}_5 \text{O}_2 + 3\text{H}_2\text{O}$ . . . . .	2.4582
Potassium Benzoate, anhydrous, $\text{K C}_7 \text{H}_5 \text{O}_2$ . . .	1.8378
Potassium Bitartrate, $\text{K H C}_4 \text{H}_4 \text{O}_6$ . . . . .	2.1582
Potassium Citrate, cryst., $\text{K}_3 \text{C}_6 \text{H}_5 \text{O}_7 + \text{H}_2\text{O}$ . . . . .	2.1405
Potassium Citrate, anhydrous, $\text{K}_3 \text{C}_6 \text{H}_5 \text{O}_7$ . . .	1.1719
Potassium Lactate, $\text{K C}_3 \text{H}_5 \text{O}_3$ . . . . .	1.4706
Potassium Salicylate, $\text{K C}_7 \text{H}_5 \text{O}_3$ . . . . .	2.0215
Potassium and Sodium Tartrate, cryst., $\text{K, Na C}_4 \text{H}_4 \text{O}_6 + 4 \text{H}_2\text{O}$ . . . . .	1.7842
Potassium and Sodium Tartrate, anhydrous, $\text{K Na C}_4 \text{H}_4 \text{O}_6$ . . . . .	1.3285
Potassium Tartrate, cryst., $\text{K}_2 \text{C}_4 \text{H}_4 \text{O}_6 + \frac{1}{2} \text{H}_2\text{O}$ . . . . .	1.3198

Potassium Tartrate, anhydrous, $K_2 C_4 H_4 O_6$	1.2982
Sodium Acetate, cryst., $NaC_2 H_3 O_2 + 3H_2O$	1.9155
Sodium Acetate, anhydrous, $Na C_2 H_3 O_2$	1.1546
Sodium Benzoate, $Na C_7 H_5 O_2$	2.0277
Sodium Bitartrate, $Na H C_4 H_4 O_6 + H_2 O$	2.6756
Sodium Citrate, cryst., $Na_3 C_6 H_5 O_7 + 2H_2 O$	1.3800
Sodium Citrate, anhydrous, $Na_3 C_6 H_5 O_7$	1.2109
Sodium Lactate, $Na C_3 H_5 O_3$	1.5773
Sodium Salicylate, $Na C_7 H_5 O_3$	2.2530
Sodium Tartrate, cryst., $Na_2 C_4 H_4 O_6 + 2H_2 O$	1.6193
Sodium Tartrate, anhydrous, $Na_2 C_4 H_4 O_6$	1.3357

## AMMONIA

618. **Determination of ammonia** is customarily made by distilling with an alkali hydroxide, receiving the distillate in a measured volume of volumetric acid and titrating the residual acid with volumetric alkali, using methyl orange, methyl red or rosolic acid as indicator.

619. **Formaldehyde method of assay.** Formaldehyde reacts with ammonium compounds to form hexamethylene tetramine, setting free an amount of acid equivalent to the ammonia present. The solution of ammonia or of an ammonium salt must be rendered exactly neutral, and the formaldehyde solution also must be neutral, the indicator employed being in both cases methyl orange. If carbon dioxide is present, it must be removed by boiling. To the cold solution add the formaldehyde solution in excess and after 12 hours titrate the mixture with volumetric sodium hydroxide, using phenolphthalein as indicator. Each mil of decinormal alkali corresponds with  $N H_4$  0.001703 gm. or with  $N$  0.001401 gm. If the ammonia is present partly free, partly in the form of a non-volatile salt, the total ammonium is to be determined as above while a second sample is to be boiled to expel free ammonia and the combined ammonia is to be determined in the residue, the free ammonia being found by difference. Since most

ammonium salts are easily dissociated, very exact results are not to be expected in this determination of combined ammonia.

**620. The standards of the U. S. P.** for salts of ammonia (with exception of the carbonate) are based on the quantity of the acid radical rather than the base. Since the salt is required to be practically wholly volatile and not more than slightly acid to litmus, this method is as good as any. It is, however, very easy to determine the ammonia by the foregoing method and base the standard on this determination. The titration equivalents for the principal official ammonium salts will be, for each mil of decinormal alkali consumed:

Ammonia ( $NH_3$ )	0.001703
Ammonium Acetate	0.007707
Ammonium Benzoate	0.013908
Ammonium Bromide	0.009796
Ammonium Carbonate (U. S. P.)	0.005494*
Ammonium Chloride	0.005350
Ammonium Citrate	0.008106
Ammonium Hypophosphite	0.008310
Ammonium Iodide	0.014496
Ammonium Salicylate	0.015508

## ANTIPYRINE

**621. Determination of Antipyrine by precipitation as an iodine compound** has been attempted with indifferent success by Manseau (1889), Schuyten (1895) Kippenberger (1896), Bougault (1898) and Zernik (1906). Bougault's method was based on the observation that one molecule of antipyrine dissolved in alcohol containing mercuric chloride absorbs one molecule of iodine. His procedure was as follows: Dissolve 0.2 gm. of antipyrine in 20 mils of alcohol, add 20 mils of an alcoholic solution (2.5 percent) of mercuric chloride and titrate with an alcoholic solution of iodine (1.351 gm. in 100 mils) until a permanent yellowish coloration appears. If the antipyrine

\*Each mil corresponds with 0.001703 gm.  $NH_3$ , the standard being not less than 30 nor more than 32 percent of  $NH_3$ .



is pure, exactly 20 mls of the iodine solution will be required.

**622. Method I** of W. O. Emery and S. Palkin\* is based on the fact that in a neutral or slightly alkaline solution, the iodine precipitate formed consists of iodoantipyrine ( $C_{11}H_{11}N_2IO$ ), this compound being easily extracted from aqueous mixtures by shaking out with chloroform. The antipyrine may be commercially pure, or it may be mixed with other substances provided only that these do not yield after treatment with iodine anything which chloroform will extract from the solution. To carry out the assay, dissolve a weighed portion of the sample, which should not contain more than 0.25 gm. of antipyrine, in 20 mls of water, and transfer to a separator, rinsing the container with several portions (in all 10 mls) of a solution containing 0.5 gm. of sodium bicarbonate. Add 5 mls of chloroform (washed with water to free it from alcohol) followed by tenth-normal volumetric iodine solution, added gradually with constant vigorous shaking until the iodine is in distinct excess (30 to 40 mls of the volumetric solution will generally be required). Shake the mixture at intervals during five minutes, then add, drop by drop, a solution (1:5) of sodium thiosulphate until the iodine color is just discharged. Shake out the solution with three portions of chloroform (25 mls each), washing each portion in a second separator with 5 mls of water and filtering through a small dry filter into a tared beaker. Evaporate the chloroform by aid of an air current or a gentle heat, dry the residue at  $110^\circ$  one-half hour, cool and weigh. Multiply the weight by 0.5991 to obtain the weight of antipyrine in the sample.

**623. Method II**, applicable where the antipyrine is not mixed with any substance yielding with iodine a precipitate insoluble in an acid-aqueous mixture. Dissolve the weighed sample, which must not contain more than 0.25 gm. of antipyrine, in a 500 ml Erlenmeyer in 50 mls of water, add 15 mls of hydrochloric

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\*Journ. Ind. and Eng. Chem., Sept. 1914, 751-3.



acid (32%) and 50 to 60 mils of tenth-normal iodine solution, shake well and allow the tarry precipitate to settle completely by standing over night. Decant the clear solution through a funnel plugged with glass wool, overlaid with a little asbestos. Wash the precipitate 8 or 10 times with 20 mil portions of 5 percent hydrochloric acid, twirling the flask vigorously, but retaining as much of the tarry precipitate as possible in the flask. Finally transfer the precipitate to the funnel. Dissolve the portion which adheres to the flask in 20 mils of warm methyl alcohol (free from ethyl alcohol or acetone), which is then used to dissolve the precipitate in the funnel, the solution being transferred to a 250 mil separator. Wash flask and filter with several small portions of methyl alcohol, until the solvent passes free from color, using in all perhaps 50 mils. Add 5 gm. of sodium bicarbonate and 50 mils of water and shake vigorously at intervals during five minutes. Remove excess of iodine by a few drops of solution of sodium thiosulphate (1 : 5) and extract the iodoantipyrine with chloroform precisely as prescribed in Method 1.

**624. Antipyrine produces with picric acid\*** a precipitate very sparingly soluble in water. In case of a solution neutral in reaction, add a decided excess of twentieth-normal solution of picric acid and determine the excess by titration of an aliquot portion of the filtrate with volumetric solution of potassium hydroxide, using phenolphthalein as indicator. Otherwise the precipitate may be employed for an approximate gravimetric determination. Dissolve about 0.5 gm. of the sample, accurately weighed, in 50 mils of water, add 5 mils of normal hydrochloric acid and heat the mixture to boiling. Add 10 mils of a cold saturated solution of picric acid in alcohol, leave the mixture at rest six hours. Collect the crystalline precipitate on a filter, wash it with a saturated aqueous solution of picric acid, dry at 95° C. and weigh. Multiply by 0.45 to find approximately the weight of antipyrine.

\*Assay method of Lemaire; Pharm. Journ., Jan. 7, 1905, 13, from Repertoire, 1904, 16, 493.

**625. Silicotungstic acid precipitates antipyrine** quantitatively from solutions acidified with hydrochloric acid. The precipitate may be collected, washed, dried and ignited for determination of the base, 0.1 gm. of the ignited residue corresponding to 0.033174 gm. of antipyrine.

**626. Determination of antipyrine in mixture with caffeine** (Emery and Palkin\*). The antipyrine is precipitated, following the details of (622), as iodoantipyrine mixed with the caffeine as alkaloid. Dissolve the weighed residue in 5 mls of glacial acetic acid, add 10 mls of a saturated solution of sulphurous acid, transfer to a large beaker, make up to 200 mls with water and add sufficient silver nitrate test solution to precipitate all the iodine. Acidify with nitric acid, collect the silver iodide on a tared filter, wash with hot water, then with alcohol, dry at 110 C. and weigh. To find weight of antipyrine in the sample, multiply weight of silver iodide by 0.8012. For the weight of the precipitated iodoantipyrine, multiply weight of silver iodide by 1.3374. Deduct this from the weight of the residue from chloroform solution of (622) to obtain quantity of caffeine present in the sample.

**627. Assay by potassium-bismuth iodide** (Thoms†). One gramme of the sample is dissolved in 50 mls of 10 percent sulphuric acid and solution of potassium-bismuth iodide (96) is added in sufficient excess. The precipitate is collected on a filter, washed with acidulated water and the filter with the moist precipitate is introduced into a stoppered flask with 10 gm. of sodium carbonate and 30 mls of 10 percent solution of sodium hydroxide, and shaken continuously one hour. The liberated antipyrine is shaken out with chloroform, the solvent evaporated and the residue dried and weighed.

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\*Journ. Ind. and Eng. Chem., July 1915, 519.

†Ber. D. Pharm. Ges., 1906, No. 4.

## ANTIPYRINE SALICYLATE (SALIPYRIN)

628. Assay of this product is made according to the Swiss Pharmacopœia by dissolving the sample in diluted alcohol and titrating the solution with tenth-normal sodium hydroxide; using phenolphthalein as indicator, thus determining the salicylic acid. Each mil of the volumetric alkali corresponds with 0.013805 gm. of salicylic acid (or 0.032617 gm. of antipyrine salicylate if this is pure.) The antipyrine is determined by shaking out from the solution after titration, with several successive portions of chloroform, evaporating off the chloroform, taking up the residue with ether, evaporating once more, and finally drying to constant weight and weighing (verifying also the melting point which should be about  $112^{\circ}$  C.) Pure dry antipyrine salicylate contains 42.325 percent of salicylic acid and 57.675 percent of antipyrine. The Swiss Pharmacopœia requires at least 42.3 percent of the former and not less than 57 percent of the latter.

## BENZALDEHYDE

629. Among the reactions which offer a basis for an assay of benzaldehyde none seems more promising than that with an alkali hydroxide, resulting in the formation from two molecules of benzaldehyde of one of alkali benzoate. According to F. D. Dodge\* the reaction takes place quantitatively when the strength of the alcoholic solution (of potassium hydroxide) is four-tenths normal. Details of his mode of procedure are not at hand, but obviously the quantity of benzaldehyde is to be calculated from the reduction in alkalinity of the mixture, each mil of four-tenths normal alkali corresponding with 0.02121 gm. of benzaldehyde.

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\*Orig. Com., 8th Internat. Cong. Appl. Chem., 17, 15.

630. The reagent, however, which is now most commonly used is **phenylhydrazin**, which throws down quantitatively a precipitate practically insoluble in ten percent alcohol\*. The assay has taken several forms.

631. (1) **For determination of small quantities** of benzaldehyde, e. g. in kirschwasser†, the precipitate of phenylhydrazone is simply collected on a tared filter, dried and weighed. In its most recent form: the assay is carried out in the following manner‡. Take for the experiment a quantity of the sample containing 10 to 100 mg. of benzaldehyde. Dilute with water to a volume of 140 mls and distil off 110 mls. Ascertain by pycnometer or refractometer approximately the quantity of alcohol. Transfer to a 300 mil Erlenmeyer flask an aliquot portion (approximately 100 mls) of the distillate and add water or alcohol as the case may be to bring the alcoholic strength approximately to 10 percent by volume.

632. Add 10 mls of a solution prepared by mixing 3 mls of glacial acetic acid with 40 mls of water, adding 2 gm. of pure phenylhydrazine, shaking thoroughly and filtering through several thicknesses of filter paper. The solution must be used immediately after filtration. Stopper the flask with a rubber stopper and shake vigorously and continuously for ten minutes. Collect the precipitate carefully in a tared Gooch crucible. Wash with cold water, finishing with about 10 mls of 10 percent alcohol. Dry in a vacuum desiccator 20 to 24 hours, or in a vacuum oven at 70° to 80° C. for 3 hours—in either case in the dark. Meanwhile a blank is to be run for a correction to the weight of the precipitate, such correction being usually about 0.5 mg. Multiply the corrected weight of the precipitate by 0.5411 to find the weight of the benzaldehyde in the aliquot taken for assay. The operation must be carried through

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\*Fischer in Zeitschr. anal. Chem., 1885, 25, 230.

†Cuniasse and Raczkowski, in Monit. Scient., 1895, 917.

‡Woodman and Davis, in Journ. Ind. and Eng. Chem., 1912, 388-9.

expeditiously to avoid loss of benzaldehyde by oxidation. The result is likely at best to be from 3 to 5 percent low.

**633. (2) Method proposed by Benedikt and Strache** as a general method of determining the aldehyde and ketone content of volatile oils.\* The sample is treated with a weighed quantity of phenylhydrazine, the precipitate is filtered out and the residual phenylhydrazine is decomposed by boiling with Fehling's solution, whereby the nitrogen it contains is liberated, its volume indicating the amount of unchanged phenylhydrazine.

**634. (3) Method of U. S. P. IX.** The reaction between phenylhydrazine and benzaldehyde results in a loss of alkalinity which is proportioned to the quantity of benzaldehyde. Three mls of freshly redistilled phenylhydrazine are dissolved in 60 mls of alcohol, and the alkalinity of 25 mls of the reagent thus prepared is at once determined with half-normal hydrochloric acid, methyl orange being used as indicator. One ml of the sample, accurately weighed, is treated with 25 mls of the freshly prepared phenylhydrazin reagent. At the end of 30 minutes a drop of methyl orange indicator is added, followed by a measured excess (A) of half-normal hydrochloric acid. The precipitate is collected on a filter and washed until the washings no longer redden litmus. The united filtrate and washings are titrated with half-normal potassium (or sodium) hydroxide. The amount of the volumetric solution used is subtracted from the quantity of half-normal acid (A) and the difference is deducted from the quantity of half-normal acid used in titrating the 25 mls of phenylhydrazin solution. The remainder multiplied by 0.053 gives the weight of the benzaldehyde in the sample assayed.

**635. Note.** Unless preserved out of contact with free oxygen (atmospheric air) benzaldehyde is gradually converted into benzoic acid, so that a preparation

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\**Monatschr. f. Chem.*, 14, p. 270, cited by Gildemeister and Hoffmann in "The Volatile Oils."

which is of standard strength when first tested very soon becomes sub-standard unless air is excluded.

636. **An impurity frequently present** in synthetic benzaldehyde and very objectionable on account of its odor and taste, is recognized by the tests provided in the U. S. P. for excluding chlorinated compounds. Quantitative tests for these have been devised, but are hardly necessary.

637. For determination of benzaldehyde in **flavoring extracts of bitter almond**, Woodman and Lyford\* propose a colorimetric test as follows; Prepare a fuchsin decolorized solution by dissolving 0.5 gm. of pure fuchsin in 100 mls of water and adding a solution containing 20 gm. of sulphur dioxide. When decolorized, make up the solution to 1 liter. The solution must be used within ten days after it is prepared. Alcohol strictly free from aldehyde is also required and is prepared by mixing with 1 liter of cologne spirit a solution of 1.5 gm. of silver nitrate in 3 mls of water and then adding slowly, without stirring, a solution of 3 gm. of potassium hydroxide in 15 mls of warm cologne spirit. After 12 hours the filtered spirit is distilled, 25 gm. of meta-phenylenediamine hydrochloride is added, a "fairly rapid" current of air is drawn through the solution for 3 hours, and the alcohol is finally redistilled, rejecting the first 300 mls.

638. **The assay is carried out as follows:** Dilute 10 gm. of the flavoring extract with aldehyde-free alcohol to 50 mls. Dilute further 2 mls of this solution to 20 mls with aldehyde-free alcohol in the cylinder of a colorimeter. For comparison prepare three standard solutions containing respectively 2, 4 and 6 mg. of pure benzaldehyde in 20 mls of aldehyde-free alcohol. The temperature of the solutions is brought to 15° C. and to each is added 20 mls of the fuchsin solution, also at 15° C. After 10 minutes (at 15° C.) the color of the unknown sample is matched with the nearest standard in the usual manner. (No account is taken in this assay of the aldehyde which must be present in the flavoring extract and may sensibly vitiate the result.)

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\*Journ. Am. Chem. Soc., Oct. 1908, 1607-11.

## BENZOATES AND SALICYLATES

639. **Determination of Benzoic or Salicylic acid** in alkali salts can be made quickly and with a fair degree of exactness\* by dissolving the salt (about 0.25 gm., accurately weighed) in distilled water 25 mls, adding 2 mls of normal sulphuric acid and shaking out the liberated acid with 3 or 4 portions (20, 15, 15, 10 mls) of a mixture of chloroform 2 volumes, ether 1 volume. Pass the successive portions of chloroform-ether through a second separator containing 10 mls of distilled water, unite them in a small flask, add 10 mls of distilled water and titrate with tenth-normal sodium hydroxide using phenolphthalein as indicator. Each ml of the tenth-normal alkali corresponds with 0.012205 gm. of benzoic or 0.013805 gm. of salicylic acid. The residual acid fluid in the separator may also be titrated with tenth-normal alkali, the wash water in separator No. 2 having been added. Subtract the result from 20 and multiply by the appropriate factor. See (614). Another plan is to evaporate the acid solution (with washings) in a platinum crucible to dryness and ignite, determining the alkali as sulphate (616). This will give us a line on the purity of the alkali base, showing e. g. the presence of potassium in a lithium salt.

## BROMOFORM

640. **The purity of bromoform** is perhaps sufficiently established by its physical characters, more particularly its high specific gravity. The official product contains "about" 4 percent of dehydrated alcohol, and has a sp. gr. of 2.595 to 2.620. A difference in alcohol content of one percent will change the sp. gr. by about 0.042. Determination of bromoform, in absence of chloroform or of other chlorine or bromine compounds, may be made by the method em-

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\*J. W. Ehman in Journ. Am. Pharm. Assoc., 1903, 156-7.



ployed in determining chloroform, viz. by heating the solution with an alcoholic solution of sodium hydroxide, resulting in the formation of 3 molecules of sodium bromide from each molecule of bromoform. [See (668)]. Add to the solution a measured excess of tenth-normal silver nitrate solution and titrate excess of silver with tenth-normal potassium sulphocyanate. Each mil of tenth-normal silver nitrate consumed in the titration corresponds with 0.0084257 gm. of C H Br<sub>3</sub>. However, the important question is not exactly how much absolute bromoform is present, but rather whether the preparation is free from impurities having possibly dangerously poisonous properties, and that question chemistry is not at present in position to answer.

## CAMPHOR

**641. An exact quantitative determination of camphor** is not easily made. Approximate results, however, suitable for standardizations of such U. S. P. preparations as spirit of camphor or liniment of camphor, may be reached by methods easily carried into effect. The plan which is most commonly adopted in the case of spirit of camphor is to throw the camphor out of solution by addition of a strong aqueous solution of some mineral salt and then dissolve it in a weighed or measured portion of some such immiscible solvent as petroleum benzin, noting the increase in weight or volume.

**642. I. Penniman and Randall\***, who apply the method also to determinations of certain volatile oils in alcoholic solution, proceed as follows; introduce into a Babcock milk testing bottle 5 mls, accurately measured, of the spirit, fill the bottle nearly to the neck with a clear solution of calcium chloride, sp. gr. 1.37, add exactly 1 mil of petroleum benzin (gasoline), boiling point 40° to 60° C., shake, fill with the same saline solution to near the top of the graduated scale, stopper securely, shake violently and whirl in the

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\*Journ. Ind. and Eng. Chem., 1914, 926.

centrifuge at high speed five minutes. If the salt solution is still cloudy, shake once more and whirl again. Read the volume of the gasoline measured from the lowest point of the lower meniscus to the extreme edge of the upper, each unit equal to 0.2 mil. From this subtract 5 and multiply the remainder by 4 to find the percentage of camphor in the spirit. (In this calculation, the specific gravity of camphor appears to be taken as practically 1.00 instead of about 0.99—a difference, however, that could hardly be distinguished by the reading of the Babcock scale. The percentage found is on the weight-volume basis. In absence of a Babcock outfit, a cassia flask may be used for the test, *mutatis mutandis*.

643. II. The older method of Arnost\* is similar in principle, but makes use of a piece of apparatus constructed especially for this purpose. Diluted sulphuric acid about 0.6 percent, colored red with fuchsin is used in place of the calcium chloride solution, 90 mls of this being used, with 10 mls of the spirit of camphor and 50 mls of petroleum benzin, sp. gr. 0.60 to 0.67. The liquids are shaken together vigorously two minutes, then left 30 minutes or more to separate, at a fixed temperature of 15° C. The gain in the volume of petroleum benzin solution corresponds with the quantity of camphor which has been taken up by that solution, each mil representing a weight of 1.0074 gm., the percentage (by weight) to be deduced from the specific gravity of the spirit, or else the result to be stated in centigrams of camphor per mil of the spirit, in conformity with the usual American practice. Allowance must be made for condensation on mixing the spirit with water. This will amount to about 0.6 degrees on the graduated scale, to be taken as an additive correction.

644. III. A simple and very practical method of testing spirit of camphor is that proposed by James Seymour†. Introduce into a narrow, accurately

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\*Zeitschr. f. Unters. d. Nahrungen, 1906, No. 9. ‡Proc. Am. Pharm. Assoc., 1907, p. 866 (cut of apparatus).

†Proc. Am. Pharm. Assoc., 1907, 443-4.

graduated cylinder exactly 10 mls of the spirit and 0.1 gm. of well dried potassium carbonate and shake well. This should not liquify or adhere to the bottom of the cylinder, otherwise the alcohol is not of full strength. If it does liquify, continue to add dried potassium carbonate, about 0.05 gm. at a time, until a portion remains undissolved after some minutes shaking. When the liquids have completely separated note the volume of the alcoholic solution. Subtract this from 10 to find the quantity of water in the spirit. Test a second sample (10 mls) of the spirit for its camphor content by placing it in the graduated cylinder, adding a volume of alcohol equal to that of the water present, followed by 2 gm. of crystallized chloral hydrate. When this is dissolved, add water to make up a volume of 80 mls, shake thoroughly and set by to allow the chloral-camphor to settle. The volume of the separated chloral-camphor should be 2 mls indicating that the spirit contains 10 percent (weight-volume) of camphor. If the quantity of camphor is less than 2 percent, there will be no separation. To find approximately the percent of camphor, multiply the volume in mls of the chloral-camphor by 4 and add 2. Thus a volume of 1.25 mls will indicate  $(1.25 \times 4) + 2 = 7$  percent. A constant temperature must be maintained throughout the tests, about 20° C. being generally most convenient.

645. IV. **The following seems the simplest** and perhaps the best plan for testing spirit of camphor. It is assumed that the alcohol and camphor are free from impurities. Take the specific gravity of the sample at 15.56° C., water at the same temperature taken as 1.000. It should be close to 0.834. If higher than this, water is probably present, if lower there must be a deficiency in the amount of camphor. Add to 25 mls of the sample, accurately measured, one gm. of anhydrous potassium carbonate and shake at intervals during half an hour. If there is no apparent change in the potassium carbonate indicating presence of water, take the specific gravity once more. It should not show appreciable change (proof that the alcohol is of full official strength). If the potassium

carbonate has shown unmistakable signs of the presence of water, do not take the specific gravity, but add 4 gm. more of the salt—even more if necessary for complete dehydration—shake at intervals during half an hour, decant the spirit carefully, avoiding loss, into a 25 mil measuring flask. Wash the residual salt repeatedly with small portions (1 or 2 mls) of 95 percent alcohol, adding the washings to the measuring flask until this is filled to the mark. Now take the specific gravity which should be close to 0.834. If lower than this, subtract from it 0.816 and divide the difference by 0.0018 to find approximately the percent (weight-volume) of camphor present in the spirit.

646. The percent of alcohol in the menstruum may be ascertained approximately by aid of an alcohol table after subtracting from the specific gravity at 15.56° C. the increased weight due to the camphor. If the spirit contains in 100 mls 10 gm. of camphor, this correction will be  $0.018(S - 0.816) \div 8.25$ , S standing for the specific gravity of the spirit at 15.56° C. If the sample has been found to contain less than 10 gm. in 100 mls, the above correction is to be multiplied by one tenth of the number of gm. in 100 mls, ascertained in the foregoing paragraph.

647. Specific gravity (approx.) of solutions of Camphor in official alcohol.

Gm. of Camphor in 100 mls.	Sp. Gr. of spirit 15.56°/15.56° C.
1	0.8179
2	0.8197
3	0.8216
4	0.8234
5	0.8253
6	0.8271
7	0.8290
8	0.8308
9	0.8327
10	0.8345

8.179  
0.018  
7

Note that a difference in sp. gr. of 0.0001 corresponds with a difference in percentage strength (weight-volume) of about 0.054.

648. V. A simple test which is especially suited to the needs of the practical pharmacist is that of

Bataille\*, who adds to 10 mls of the spirit with constant shaking distilled water until a permanent precipitate is produced. The quantity required in the case of the preparation of the French Codex he found to be exactly nine mls. Both the German and the Swiss pharmacopoeias have made official this method of testing, the latter requiring that to produce a slight permanent precipitate in 10 gm. of the spirit at 15° C., not less than 4.6 nor more than 4.8 mls of water at the same temperature shall be required. L. D. Havenhill and F. E. Rowland† found that in 5 mls of spirit of camphor U. S. P., precipitation was caused at 20° C. by 4.7 mls of water, provided the spirit were of official strength, a difference of 0.1 mil corresponding (within a narrow range) to a difference of nearly one third of one percent (weight-volume) in the camphor content of the spirit. If the spirit has the official specific gravity and stands the U. S. P. test for water with potassium carbonate (assuming of course that the alcohol itself is free from impurities such as acetone or methyl alcohol) incipient precipitation should require at 20° C. not less than 4.6 nor more than 4.8 mls of water.

649. Quantity of water required to cause a permanent precipitate at 20° C. in spirit of camphor made with official alcohol, as proved by the potassium carbonate test.

Gm. Camphor in 100 mls of spirit	Mils of Water required to cause turbidity in 5 mls of the spirit
4	7.90
5	6.95
6	6.40
7	5.90
8	5.45
9	5.05
10	4.70
11	4.40
12	4.15
13	3.92
14	3.70
15	3.50

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\*Bull. Sei. Pharm., 1913, 19, 409.

†Journ. Am. Pharm. Assoc., 1912, 590-3.

650. **VI. Direct weighing of the camphor** is the distinctive feature of the method of Jumeau\*. To 10 mls of the spirit add 40 mls of the official solution of lead subacetate and shake well. Collect the precipitated camphor on a filter, wash it with a little water, dissolve it in a minimum quantity of ether and evaporate the ethereal solution in a tared beaker by a current of warm air, taking care that atmospheric moisture is not condensed by the cold produced in the evaporation. Drying is completed in the desiccator, and the camphor is weighed. Of course very exact results cannot be hoped for.

651. **VII. The method may be improved** by bringing the precipitated camphor into solution in a non-volatile oil, weighing the solution, driving off the camphor by heating at  $110^{\circ}$  C. and weighing the oil once more. The camphor may be precipitated with a saline solution (or as above, with solution of lead subacetate), collected on a filter and washed with a little cold water. When the filter is well drained, transfer the moist precipitate to a small mortar containing dried sodium phosphate, add 25 mls of a suitable fixed oil, triturate until all moisture has been taken up by the sodium phosphate; treat the filter itself in a similar manner, to bring into solution in oil the camphor adhering to it. Transfer the oil to a shallow evaporating dish or beaker, rinsing the mortar with more of the oil, and squeezing the oil from the filter, which also is to be treated with a little more of the oil. Weigh the oily solution, which will contain some sodium phosphate, heat one hour in a shallow dish at  $110^{\circ}$  C. to drive off camphor, cool and weigh the residual oil. See (655).

652. **VIII.** There is no wholly satisfactory method of determining camphor by chemical means. By reaction with hydroxylamine, camphor oxime is produced, but not strictly quantitatively, although H. C. Fuller† has based on this reaction a method of assaying spirit of camphor.

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\*Bull. de Science Pharmacol; 1913, No. 10.

†Pharm. Journ. and Pharmacist, Sept. 16, 1911, 381.

653. **IX. In the absence of fraudulent additions,** accurate determination can be made of camphor in spirit of camphor by use of the polariscope. It is this method which has been adopted in U. S. P. IX, as also in the British Pharmacopoeia 1914. The latter authority gives the specific gravity of the spirit, which is made with 90% alcohol, as 0.845 to 0.850 at 15.5° C., the optical rotation at 15.5° as not less than + 4°. The U. S. P. uses alcohol of 94.9 percent, but does not state the specific gravity of the product, which will be in the neighborhood of 0.8345 at 15.56° C. A test is however provided (addition of dry potassium carbonate) by which a product made with alcohol not of full strength is excluded. The content of camphor is determined in the following manner: The specific rotation of a sample is taken with a polariscope. Another sample is evaporated to dryness and the camphor from it is sublimed. An alcoholic solution is made from a portion of this sublimed camphor of the same strength as the official spirit, and the specific rotation of this is also taken. Then by a simple proportion the quantity of camphor in the original spirit is deduced.

654. It is to be noted that no **standard of specific rotation** is prescribed in the U. S. P. IX, so that apparently synthetic camphor might be employed as well as natural camphor, but such a deduction would be fallacious, since the method obviously is not applicable in case the spirit shows no specific rotation. If the intention is to exclude synthetic camphor, it will be necessary to fix maximum and minimum limits for the specific rotation of the spirit.

655. **Determination of camphor in the official liniment of camphor.** This would seem to be a very simple problem. C. O. Miller\* directs to evaporate the camphor from a weighed quantity of the liniment in a flat bottom dish by heating at 110° C. for 90 minutes. The loss in weight is assumed to be due wholly to volatilization of camphor, and such an assumption is generally safe. Actual gain in weight

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\*Journ. Am. Pharm. Assoc., 1915, 683.

from oxidation is a possibility also not to be overlooked. It would be well to guard against presence of moisture by filtering the liniment through dry paper, before weighing the sample.

656. **Kebler and his associates\*** propose as an alternative method, determination of the optical rotation of the liniment in comparison with that of a sample of liniment prepared, with precaution against any loss of camphor by volatilization, in accordance with the U. S. P. formula. Such a sample they found to produce a rotation of  $+58.5^\circ$  on the sugar scale. If the sample under examination produces a rotation different from this, the quantity of natural camphor present will be found by a simple proportion. In order to make the result convincing and complete, it is necessary to be assured that the solvent itself produces no specific rotation. Synthetic camphor of course is ruled out by this assay method.

657. **The method of the U. S. P. IX** is similar to that for spirit of camphor and open to the same criticisms.

658. **Determination of camphor in tablets, etc.** The method proposed by E. Dowzard† is simple and generally applicable. The apparatus required consists of (1) a flask for generating steam, (2) a second flask to contain the material from which camphor is to be extracted by the steam and (3) a receiver of special pattern in which the steam is condensed by application to its surface throughout of cold water. The camphor is extracted from the distillate by shaking with benzene, and the amount of natural camphor is determined by the polariscope. When 25 mls of benzene are used and the rotation taken in a 100 mm. tube, the author found that each minute of rotation corresponded with 0.01001 gm. of camphor, but it is advised that the factor be determined by actual experiment for the particular piece of apparatus used. The same method could obviously be employed for

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†Journ. Am. Pharm. Assoc., 1917, 617-8.

\*Journ. Ind. and Eng. Chem., 1914, 489, 90



the official liniment of camphor or for ointments and similar preparations that do not contain other volatile substances.

659. Query: would not maceration with benzene followed by distillation, or maceration with a suitable non-volatile solvent, followed by distillation with benzene vapor furnish a solution suitable for polariscopic examination? Also would such a solution have any advantage over an alcoholic solution prepared in a similar manner?

### CAMPHOR MONOBROMATED

660. When a solution of monobromated camphor in toluol is treated with metallic sodium, the bromine unites with the metal, forming quantitatively sodium bromide. It is a simple matter therefore to determine the proportion of true monobromated camphor contained in a given sample which answers in physical characters to the U. S. P. description. Following the method of André and Leulier\*, put into a 125 mil distilling flask 10 gm. of toluol, 0.5 gm. of monobromated camphor and about one gm. of metallic sodium. Heat under a reflux condenser one hour. After cooling, add 30 mls of water, acidulate the mixture strongly with nitric acid, add 25 mls of tenth-normal silver nitrate solution and 2 mls of ferric ammonium sulphate test solution. Finally titrate the excess of silver with tenth-normal potassium sulphocyanate, subtract the quantity in mls of this reagent consumed from 25 and multiply the remainder by 0.023104 to find the quantity of monobromated camphor in the sample.

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\*Journ. Pharm. Chim., 1910, 2, 64-6.

## HYDRATED CHLORAL

**661. Caustic alkalis decompose hydrated chloral** rapidly, chloroform and formic acid resulting from the reaction. The latter combines with the alkali so that if excess of a volumetric solution of the alkali is used, residual titration will show the quantity of acid produced and so by a simple calculation the quantity of hydrated chloral in the solution. The reaction at the outset is strictly quantitative, but a secondary reaction occurs slowly between the chloroform and the excess of alkali by which a portion of the latter will be converted into chloride. At ordinary room temperatures this secondary reaction may be ignored if the residual titration is made at once. J. Garnier\* gives the following directions for the assay; Dissolve 1 gm. of the sample in 50 mls of water and add 75 mls of tenth-normal potassium hydroxide solution. Let the mixture stand 15 to 20 minutes, at a temperature not exceeding 15° C. Then promptly titrate the excess of alkali with tenth-normal sulphuric acid, using phenolphthalein as indicator. Subtract the quantity in mls of volumetric acid used from 75 and multiply the remainder by 0.01654 to find the quantity in grammes of hydrated chloral in the sample tested. A second test should be made in which only a very small excess of volumetric alkali is to be used. Care should be taken of course that the solution of hydrated chloral is strictly neutral. It is well to add to it a drop of phenolphthalein indicator and neutralize if necessary before adding the volumetric alkali. The U. S. P. IX employs this assay, using, however, about 4 gm. of the sample, with alkali and acid of normal strength. Nothing is said of the temperature, but the time allowed for the reaction is only two minutes, so that the result cannot be appreciably affected by the secondary reaction.

**662. Lime water can be substituted** for the volumetric alkali with advantage since this does not

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\*Bull. Sci. Pharmacoll., 1908, 15, 77-82.

react with chloroform. Use 100 mls of the lime water the strength of which must be found by titrating an equal quantity of it with decinormal acid. The quantity of hydrated chloral used should not exceed 0.5 gm., care being taken that the solution of this in water (10 mls) is strictly neutral.

663. **By prolonged heating with** an alcoholic solution of **an alkali hydroxide**, the whole of the chlorine is made to combine with the alkali; the chloride formed may then be determined with silver nitrate and so the quantity of hydrated chloral indicated. T. E. Wallis\* proceeds as follows: Dissolve 0.5 gm. of the sample in 50 mls of alcohol in a strong bottle having a well fitted ground glass stopper (pressure bottle), add 50 mls of normal sodium hydroxide solution, secure the stopper by wiring or otherwise and heat three hours in a boiling water bath. Neutralize the solution with diluted nitric acid and determine chloride in it by tenth-normal silver nitrate, one mil of which corresponds with 0.005513 gm. of hydrated chloral. Of course absence of chloride from the sample tested must be established.

664. **An iodometric assay** is carried out by E. Rupp† by mixing in a stoppered bottle a solution in 10 mls of water of 0.1 gm. of the sample with 25 mls of tenth-normal iodine solution and 25 mls of normal potassium hydroxide solution. After 5 to 10 minutes the solution is diluted with 50 mls of water, 5 mls of strong hydrochloric acid are added and the liberated iodine is titrated with tenth-normal sodium thiosulphate. Subtract amount in mls of the latter reagent consumed in the titration from 25 and multiply by 0.00827 to find the quantity in grammes of hydrated chloral in the sample.

665. **Reduction by zinc dust** is the basis for an assay proposed by P. A. W. Self‡. Boil under a reflux condenser 20 minutes a solution in 60 mls of water of about 0.3 gm. of the sample with 2 gm. of

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\*Pharm. Journ., 1906, 76, 162-3.

†Arch. Pharm., 1903, 241, 326-8.

‡Pharm. Journ., 1907 (IV) 25, 4-7.

pure zinc dust. Rinse the condenser with a little water which is added to the solution, and, while this is still hot, 10 mils of acetic acid are added. After 2 minutes, the solution is filtered and the chlorine present as chloride is determined with silver nitrate. Each mil of tenth-normal silver nitrate solution corresponds with 0.005513 gm. of hydrated chloral.

## CHLOROFORM

666. **No practical assay method** for exact determination of the percentage of  $\text{C H Cl}_3$  in commercial chloroform has yet been devised. The purity of a sample must be judged by its physical properties, after applying certain qualitative tests for traces of objectionable impurities. The specific gravity for official chloroform U. S. P. IX is permitted to have a range only from 1.474 to 1.478 at  $25^\circ \text{C}$ . This assumes the presence of 0.6 to 1.0 percent by weight of alcohol. The boiling point (U. S. P.) is about  $61^\circ \text{C}$ , a requirement, however, that is altogether too vague. Chloroform in fact, under standard conditions should not begin to boil under  $60^\circ \text{C}$ . and the temperature should not rise up to the very end of the distillation above  $62^\circ \text{C}$ . The presence in chloroform of a small proportion of alcohol, is essential to stability. The quantity should not be less than 0.5 percent nor over 1 percent. The quantity may be approximately ascertained as prescribed in the French Codex by titration with a standard solution of potassium dichromate, each mil of which corresponds with 0.004 gm. of absolute alcohol. Ten mils of the chloroform are shaken out with 3 successive portions of strong sulphuric acid (4, 4 and 2 mils). The acid is added gradually to 80 mils of water and from the mixture is distilled off 40 mils, which will contain the alcohol from 10 mils of the chloroform. Of the distillate, 10 mils are transferred to a test tube, 2 mils of strong sulphuric acid are added and the tube is immersed in a boiling water bath. The standard solution of potassium dichromate (16.97 gm. to the liter) is now added drop by drop until the blue color at first pro-

duced changes to a clear green. For each mil of the dichromate solution, reckon 0.004 gm. of absolute alcohol. The quantity corresponding with 0.5 percent should therefore be 4.61 mils.

670. To determine the quantity of **chloroform in mixtures** such as cough syrups, it is necessary first to distil off the chloroform. The distillation is facilitated by adding to the mixture before distillation a certain quantity of alcohol, generally 10-15 percent of its volume. The receiver should also contain alcohol, below the surface of which the distillate is discharged. A better plan, particularly when the quantity of chloroform is small, is to aspirate air slowly through the mixture, and collect the chloroform by aid of a condenser (or scrubber) consisting of a glass tube 1 meter long and 3 cm. wide filled with small glass beads, over which a slow current of alcohol is made to trickle. The vapors are drawn into this tube from near the bottom, the alcohol, carrying with it the absorbed chloroform, passing into a receiver kept at a low temperature. Air may be safely aspirated through the apparatus at the rate of 1.5 liters per minute. The chloroform is finally to be determined either by treatment with alcoholic solution of sodium hydroxide (663), or by decomposition by zinc dust (665). In either case 1 mil of tenth-normal silver nitrate corresponds with 0.0039797 gm. of chloroform.

671. **Details of assay of medicinal syrups** are given by E. B. Putt\*, essentially as follows: Place in a 250 mil Kjeldahl flask, 10 mils of the syrup, or a quantity containing about 0.1 mil of chloroform, add about 1 gm. of calcium carbonate (or sufficient to neutralize any free acid) and 80 mils of alcohol. Distil, using a vertical condenser having a delivery tube extending well down into a 150 mil pressure flask standing in cold water, until about 70 mils of distillate have come over. Add to the distillate 10 mils of a solution of potassium hydroxide (chloride free) made by dissolving 100 gm. of the alkali in 100 mils of water. Close the flask securely, mix the contents well and heat 1 hour in a gently boiling water bath. Cool, and

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\*Am. Food Journ., 1915, 467-8.

determine the chloride with silver nitrate volumetrically or gravimetrically. Each mil of tenth-normal silver nitrate corresponds with 0.0039797 gm. of chloroform or each gm. of silver chloride corresponds with 0.27764 gm. of chloroform. In case of lozenges and similar preparations, use for the assay about 2 gm. of the sample, dissolved in 10 mls of water or diluted alcohol, as the case may be.

## CITRAL

672. **The value of oil of lemon** for flavoring uses depends on its content of the oxygenated compounds citral and citronellal. The proportion of these is small and as yet there is no wholly satisfactory method of determining accurately the quantities of each. Several methods have been proposed which give results of value for comparative purposes.

673. **The sulphite method**, which in a modified form was official in the U. S. P. VIII, and is still employed in the determination of carvone and of cinnamic aldehyde, is not suited to the assay of oil of lemon. It may, however, be employed in the case of oil of lemon grass, in which the proportion of citral is very large, while citronellal is absent. It is not applicable to oil of citronella. The test is carried out according to A. Bloch\* in the following manner. Introduce into a cassia flask, the graduated neck of which holds fully 10 mls, ten mls (exactly) of oil of lemon grass. Add 20 mls of a freshly prepared 30 percent solution of sodium bisulphite and shake until the mixture solidifies. After 15 minutes, immerse the flask in a water bath at 85° C. and when, under constant shaking, the mass has liquefied, add sufficient of the sodium bisulphite solution to fill the flask three-fourths full. When the oily layer has separated, more of the bisulphite solution is added, little by little, until at the end of 15 minutes the oily stratum is brought into the graduated neck of the flask. The heat is continued 10 minutes longer, then the flask and

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\*Bull. Sci. Pharmacol., 1908, 15, 72-7.

contents are cooled to the same temperature at which the measurement of the sample was made and the volume of the citral is then read off in the usual manner.

**674. Phenylhydrazine assay\*.** Into a 50 mil glass stoppered flask introduce about 25 mls, accurately weighed, of oil of lemon and 12.5 mls of a freshly prepared 2 percent alcoholic solution of pure phenylhydrazine; let stand one hour, then add 25 mls of tenth-normal hydrochloric acid and mix by rotating the flask. Add 10 mls of benzene, shake vigorously and transfer to a separator. When separation is complete draw off the aqueous solution, rinse the flask twice with water (5 and 5 mls) which is used to wash the contents of the separator and then added to the solution already drawn off. (If necessary the several portions of fluid are to be filtered as they are drawn off, and the filter finally washed once with water.). To the solution add 10 drops of diethyl orange indicator (1 : 2000) and titrate to a distinct yellow color with tenth-normal potassium hydroxide. Carry out a blank assay omitting the oil of lemon. Subtract the result of the latter titration from that of the former. Each mil of the difference corresponds with 0.0152 gm. of citral in the sample taken for assay.

**675. The assay of the U. S. P. IX** is the same as the foregoing in principle, but uses as indicator methyl orange and employs semi-normal instead of tenth-normal volumetric solutions. Results of a high degree of exactness are not to be expected, but that is not of great importance since the standard is one of limitation only\*. The same method of assay may be employed for citronellal, the titration factor being changed from 0.0152 to 0.0154.

**676. Hydroxylamine assay.** The reaction of aldehydes with hydroxylamine is similar to that with phenylhydrazine, resulting in the formation quantitatively of acid, the amount of which is readily determined by titration. The assay process for citral as

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\*Modification of Dr. Kleber's method described in *Am. Perfumer*, 1912, p. 284, proposed in Schimmel's report, April 1912, p. 76.

made official in the British Pharmacopoeia of 1914 is as follows: To 20 gm. of oil of lemon contained in a distilling flask, add 20 mls of a semi-normal solution (34.751 gm. to a liter of 80 percent alcohol) of hydroxylamine hydrochloride, 8 mls of tenth-normal potassium hydroxide and 20 mls of 90 percent alcohol. Boil 30 minutes under a reflux condenser, cool and dilute with 250 mls of water, rinsing the condensing tube into the flask with part of this. Neutralize accurately with normal alcoholic potassium hydroxide, using phenolphthalein as indicator and then titrate with semi-normal sulphuric acid, with methyl orange as indicator. Run a blank omitting the oil of lemon. Subtract the quantity of volumetric acid required in the first titration from that in the blank. The difference must not be less than 10.5 mls indicating presence of not less than 4 percent of aldehydes calculated as citral.

**677. Colorimetric assay by diaminophenol hydrochloride\*.** Prepare a solution of pure citral in 50 percent alcohol (0.1 gm. in 100 mls). Measure 2 mls of this into a colorimeter tube, add 20 mls of 65 percent (vol.) alcohol and 15 mls of a reagent prepared by dissolving 0.200 gm. of diaminophenol hydrochloride (amidol) in 100 mls of 65 percent alcohol, and make up to 50 mls with 65 percent alcohol. (Preferably the alcohol used should be freed from acetaldehyde by distillation with potassium hydroxide.) Place 2 mls of the sample—flavoring extract of lemon—in the other tube with 15 mls of the above reagent and make up to 50 mls with 65 percent alcohol. Mix the contents of both tubes thoroughly, let them stand ten minutes at room temperature, then make a colorimetric comparison in the usual manner and calculate therefrom the citral percentage of the extract.

**678. Other colorimetric methods** have been proposed. Among them that of E. M. Chace† has especially attracted attention. It is essentially the

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\*L. D. Little in Journ. Am. Pharm. Assoc., 1914, 553-6.

†Journ. Am. Chem. Soc., Oct. 1906, 1472-6.



same as the method given in outline in (637) for colorimetric determination of benzaldehyde. It seems hardly necessary to give full details.

**679. Colorimetric assay by Metaphenylenediamine** of Hiltner\*, modified†. The reagent is prepared by dissolving 1 gm. of metaphenylenediamine hydrochloride and 1 gm. of crystallized oxalic acid, each in about 45 mls of 80 percent alcohol. Mix and make up to 100 mls with 80 percent alcohol. Add 2 or 3 gm. of fuller's earth, shake well, allow to settle nearly clear and decant upon a double filter. When most of the liquid has run through, add the turbid residue to the liquid in the filter. Provide a standard solution in alcohol of citral, 1 gm. to the liter. From this prepare a color standard by introducing into a 100 ml measuring flask, 4 mls of the standard solution, adding 20 mls of the foregoing reagent and filling to the mark with 94 percent alcohol. Prepare at the same time a solution of the sample, of which 5 mls are taken, with 10 mls of reagent, and make up to 50 mls. For this purpose, an orange flavoring extract is taken at full strength; of oil of orange, 4 gm. are made up to 50 mls with official alcohol; of lemon "extract" 10 gm. are diluted to 20 mls with official alcohol; of lemon oil, 0.5 gm. is made up to 50 mls in like manner.

**680.** These dilutions are used for the preliminary test. From the result of comparison of the color produced with the standard (average of 5 or more tests), a close approximation can be made to the quantity of the sample that should be used to give a color identical with that of the sample, and a second series of comparisons is made accordingly. Sometimes even a third experiment must be made before the colors match satisfactorily but the experiments consume little time, and results are said to be generally quite satisfactory. In all cases, a fresh standard color is to be prepared at the same time that the new

\*U. S. Dept. Agr. Bureau of Chemistry, Bull. 122, 34; 132, 102; 137, 70.

†C. E. Parker and R. S. Hiltner in Journ. Ind. and Eng. Chem., 1918, 608-10.

trial experiment is made. (In its original form the test was unsatisfactory because the color produced was sometimes blue instead of yellow—a difficulty overcome by addition to the reagent of the oxalic acid.)

## CINEOL

681. **Three methods, distinct in principle,** have been proposed for the determination of cineol (eucalyptol) in such volatile oils as those of cajuput and eucalyptus globulus. The first is based on the formation with certain acids of solid compounds with cineol. For the second and third, see (686) and (687). The compound with phosphoric acid was made the subject of a patent in 1894, and an assay process utilizing this reaction was deemed of sufficient merit to find a place in the U. S. P. VIII. The compound formed, however, is so unstable that it is difficult to obtain results even approaching exactness. Hydrobromic acid has been tried with similar disappointing results. In arsenic acid, however, there has been found a fairly satisfactory solution of the problem\*. Measure into a capsule of 50 mils capacity, embedded in finely cracked ice, exactly 10 mils of the oil, add 10 mils of a concentrated solution of arsenic acid, sp. g. 2.173 at 25° C., containing about 85 percent of acid, and stir until precipitation is complete. Allow the mixture to stand ten minutes longer in the ice. If the mixture tends to set into a hard mass, add 1 to 5 mils of purified petroleum benzin and mix well with the acid compound.

682. By aid of a horn spatula, transfer the compound to a hardened filter, cover with another similar filter, envelope the whole in several thicknesses of filter paper and press well one minute in a letter press. Repeat the pressing with several changes of the absorbent paper, pressing each time two minutes, until the cineol arsenate is quite dry. This operation must be conducted as expeditiously as possible to

\*J. L. Turner and R. C. Holmes in Journ. Am. Pharm. Assn., March 1915, 351-8.

avoid incipient decomposition of the acid compound. Transfer the dried arsenate to a funnel inserted into the neck of a cassia flask. By aid of a jet of hot water from a wash bottle, wash the compound into the flask, plunge the flask into boiling water and rotate until the cineol is completely separated in the form of a liquid. Finally add hot water to bring the cineol into the neck of the flask. Cool to the temperature at which the oil was measured and read off the volume of the cineol.

683. If the proportion of cineol in the oil is less than 50 percent there is difficulty in handling the precipitate. In such case use 5 mls of the oil and 5 mls of eucalyptol, deducting 5 mls from the volume of the oil measured in the cassia flask.

684. It is this assay process which has been made **official in U. S. P. IX.** The British Pharmacopoeia adheres to the phosphoric acid assay, directing in the case of oil of cajuput to mix 10 mls of the oil with 4 to 5 mls of syrupy phosphoric acid in a vessel surrounded with a freezing mixture, to press the resulting solid strongly in a piece of fine calico (i. e. muslin) between folds of blotting paper, decompose the pressed cake with warm water and measure the separated cineol in a suitable graduated tube, at  $15.5^{\circ}\text{C}$ . The requirement (B. P.) for oil of eucalyptus is not less than 55 percent (vol.); for oil of cajuput, not less than 45 percent. The U. S. P. IX fixes no standard, although it provides for the former an assay process.

685. **A modification of the U. S. P. IX assay** is here suggested as likely to yield precise as well as correct results. This consists in adding to the cineol separated from the sample a definite quantity of carbon tetrachloride, taking the specific gravity of the mixture and deducing therefrom the exact quantity of the cineol\*.

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\*Add to the cineol a sufficient quantity of carbon tetrachloride to bring the volume of the mixture at  $20^{\circ}\text{C}$ . to exactly 50 mls. Take the specific gravity at  $20^{\circ}\text{C}$ . of the mixture, also that of the carbon tetrachloride at the same temperature. Subtract the former from the latter and divide the remainder by 0.0145 to find approximately the weight in grammes of the cineol.

**686. The resorcinol assay.** In its original form\* the assay was made by simply shaking 10 mils of the sample in a cassia flask with a 50 percent aqueous solution of resorcinol. The volume of the residual oil was read off in the usual manner, the diminution in volume being taken as a measure of the cineol present. Results have been found to be erratic and often preposterously high. A modification of the method suggested by C. T. Bennett† gives results approximating the truth, but the process is not suited to the requirements of the pharmacist. It consists in fractionation of the oil to be tested, and then treating the fraction distilling between 170° and 190° with a warm 55 percent aqueous solution of resorcinol.

**687. The permanganate of potassium assay†,** depends on the fact that cineol is not oxidized by cold solution of the permanganate, while other constituents of the oil are converted into water soluble products. The results of the assay have proved inconclusive and often misleading.

## COUMARIN

**688. Detection of Coumarin in extract of Vanilla** is practiced by H. J. Wichmann§ in the following manner. Put into a separator 10 mils of the sample, add solution sodium hydroxide sufficient to render alkaline, then add 15 mils of water and shake out with 20 mils of ether. Add to the ethereal solution 3 mils of a "strong" alcoholic solution of potassium hydroxide and shake with 10 mils of water. By this procedure vanillin, saccharin and certain organic acids are removed. Put into a test tube 1 mil of a 50 percent solution of potassium hydroxide, add the washed ether solution, drive off the ether by a gentle heat, then heat the aqueous solution over a naked

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\*Schimmel & Co. Report, Oct. 1907; also Gildemeister and Hoffmann's *Volatile Oils*" 1913, p. 601.

†Chem. and Drugg., 1908, 55; Schimmel & Co. reports, April and October, 1910.

‡F. D. Dodge, in *Journ. Ind. and Eng. Chem.*, 1912, 592-3.

§*Journ. Ind. and Eng. Chem.*, 1918, 535-7.

flame. If coumarin is present a greenish yellow color appears when the last of the water is driven off, but presently disappears, indicating that the reaction is completed. Dissolve the residue in a little water, acidify the solution with sulphuric acid and shake with 10 mls of benzene; separate the benzene, wash it with water, filter and test for salicylic acid with a drop or two of ferric chloride solution. If a purple color is not produced, add a drop or two of tenth-normal sodium hydroxide, to neutralize any acid that may have been taken up by the benzene. A purple color is proof of the presence of coumarin.

689. **Determination of coumarin** in extract of vanilla. H. J. Wichmann gives the following simple procedure; to 50 mls of the extract add solution of lead acetate in slight excess and make up the volume of the solution with water to 100 mls. Filter and add to the filtrate dry potassium oxalate to remove the excess of lead. Filter once more and shake out 50 mls of the filtrate with ether, add a few drops of phenolphthalein indicator, followed by an excess of an alcoholic solution of alkali hydroxide. Separate the ethereal solution and wash it with successive 10 ml portions of water until the red color disappears. Evaporate off the ether and dry and weigh the residue as coumarin.

## ETHYL NITRITE (NITROUS ETHER)

690. **The nitrometer method of assay** has long been relied upon for determination of ethyl nitrite in the official spirit of nitrous ether. According to U. S. P. IX, a sample of the spirit (about 50 mls) is shaken with powdered potassium bicarbonate to remove any free nitrous acid. (The potassium bicarbonate should not be left in contact with the spirit more than 5 minutes, since it acts slowly on the nitrous ether, removing the combined nitrous acid.) Forty mls of the neutralized spirit are transferred to a tared 100 ml measuring flask and weighed accurately, and alcohol is then added to make up 100 mls. Ten mls of this solution are introduced into a nitrometer,

followed by 10 mls of a recently prepared 20 percent solution of potassium iodide (which should be previously boiled to expel absorbed air) and then by 5 mls of diluted sulphuric acid. After evolution of gas (nitric oxide) is complete (generally within 30 minutes) read off the volume of the gas. Multiply this volume in mls by 0.307 and divide the product by one-tenth the weight in grammes of the sample taken. The quotient, subject to following corrections is the percent of ethyl nitrite in the sample. Temperature correction is one-third of one percent of above quotient for each degree C., additive if temperature is below, subtractive if above 25° C. Barometer correction, four thirtieths of one percent of said quotient for each millimeter, additive if above, subtractive if below 760 mm. The test should be made at room temperature, not far from 25° C.

691. **The Potassium Chlorate assay** of the Dutch Pharmacopoeia is better suited to the needs of the pharmacist, and is believed to be equally reliable. To a mixture of 10 mls of distilled water and 5 mls of a cold-saturated aqueous solution of potassium chlorate, add 5 gm. of the sample (spirit nitrous ether) and 5 mls of 10 percent nitric acid. Shake the mixture (contained in a glass stoppered bottle or flask) at frequent intervals during 30 minutes, add 15 mls of tenth-normal silver nitrate and 2 mls of ferric ammonium sulphate solution (10%), and titrate the excess of silver with tenth-normal potassium sulphocyanate. Subtract the volume in mls of the sulphocyanate solution consumed from 15 and multiply the remainder by 0.022353 to find the weight of the ethyl nitrite present in the sample tested. (Make sure that the potassium chlorate is free from chloride.)

## FORMALDEHYDE

692. **Hydrogen dioxide assay** of O. Blank and H. Finkenbeiner\* modified by Haywood and Smith†. Hydrogen dioxide converts formaldehyde in alkaline solution into formic acid, the quantity of which can be determined accurately by differential alkalimetric titration. Measure into a 500 mil Erlenmeyer flask 50 mls of normal sodium hydroxide and add 50 mls of 3 percent solution of hydrogen dioxide, previously accurately neutralized with sodium hydroxide (litmus indicator). Add 3 mls, accurately measured, of the sample of formaldehyde, allowing the tip of the pipette to come quite close to the surface of the mixture in the flask. Place a funnel in the neck of the flask and set it on a steam bath, where it is to be shaken occasionally during five minutes. Remove the flask from the steam bath, wash down the funnel with distilled water, cool to near room temperature and after allowing the mixture to stand an hour or two, i. e., until gas bubbles no longer come off on shaking (Vanderkleed and E'Ve), titrate the excess of alkali with normal acid, using litmus as indicator. Subtract the quantity in mls of normal acid consumed from 50 and multiply the remainder by 0.03002 to find the weight in grammes of the formaldehyde in the sample taken, the specific gravity of which has been accurately determined so that its weight is known. (Preferably weigh the 3 mls of solution taken as in the U. S. P. assay.) Points to be particularly observed are the heating of the mixture on a steam bath, and the subsequent cooling before titration.

693. **The method prescribed in the U. S. P. IX differs from** the foregoing mainly in the order in which the reagents are added, a matter which Haywood and Smith have shown experimentally to be of consequence. The phraseology used in the U. S. P.

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\*Berichte d. Chem. Ges., 1898, 31, 2979-81.

†Journ. Am. Chem. Soc., Sept. 1905, 1183.

test, "a tared flask containing 10 mils of distilled water," is open to criticism, although the meaning can scarcely be mistaken. The "tare" is of course that of flask and water combined.

**694. Method of Hugo Schiff\***, modified by F. Herrmann†, based on that proposed by Legler‡, but substituting for a volumetric solution of ammonia, a solution of ammonium chloride, followed by volumetric sodium hydroxide. Formaldehyde combines in molecular proportions (3 : 2) with ammonia, producing hexamethylene-tetramine, a neutral compound. Weigh into a stoppered flask (150 to 200 mils capacity) about 4 mils of the sample (formaldehyde solution of about 35 percent strength), add 3 gm. of pure ammonium chloride in fine powder, dissolve the salt, neutralize accurately, then add 25 mils of double normal sodium hydroxide, the flask being meanwhile constantly shaken. Stopper the flask at once, and allow it to cool to room temperature, add then 50 mils of water and titrate the residual alkali with normal sulphuric acid, using rosolic acid (or litmus) as indicator. Subtract the quantity of standard acid used from 50 and multiply the difference by 0.045 for the weight in grammes of formaldehyde in the sample taken.

**695. The cyanide method proposed by G. Romijn§**, as modified by E. Elvove.\*\* Formaldehyde combines in equivalent proportions with alkaline cyanides to produce a compound which yields no precipitate with silver nitrate. If, therefore, a volumetric solution of potassium cyanide is added in excess to a sample of a formaldehyde solution, titration of the excess will indicate by difference the quantity of formaldehyde present. The method is of especial value in determining minute quantities of formaldehyde, accurate results being possible even where the quantity is no greater than 0.01 percent.

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\*Chem. Ztg., Jan. 1903, 14.

†Chem. Ztg., Jan. 1911, 25.

‡Berichte d. Chem. Ges., 1883, 1335.

§Zeitsch. f. anal. Chem., 1897 (36) 18, 527, 717.

\*\*Am. Journ. Pharm., Oct. 1911, 455-71.



696. The procedure is as follows: Place in a 150 mil Erlenmeyer flask 5 mls of water and weigh accurately. Add from a pipette whose point is brought quite close to the surface of the water 0.5 mil of the formaldehyde solution and weigh once more. Add immediately 100 mls of a solution of potassium cyanide (6.5 gm. to the liter), whose exact titre has been ascertained. Mix well and add to a mixture of 40 mls of tenth-normal silver nitrate with 10 mls of dilute nitric acid (10 percent) contained in a 200 mil measuring flask. Wash the Erlenmeyer flask and add the washings to the silver solution, making up the volume of this finally to 200 mls. Shake the mixture thoroughly, filter through a dry filter and titrate the excess of silver in 100 mls of the filtrate (measured after rejecting the first 25 mls that pass the filter) with tenth-normal potassium sulphocyanate, using ferric alum solution as indicator. Multiply the quantity in mls of sulphocyanate solution consumed by two and subtract from 40. The difference represents the quantity of uncombined potassium cyanide. Subtract this from the total quantity of potassium cyanide added (calculated to tenth-normal strength), multiply the difference by 0.003002 to find the quantity of HCHO contained in the sample used for the assay, from which its percentage is deduced in the usual way. The method may be employed in presence of acetone or of aldehydes other than formaldehyde. Results are uniformly good if a sufficient excess (as provided above) of sodium cyanide is used.

697. **Iodine Method of Romijn\*.** The formaldehyde is oxidized to formic acid by a measured quantity of tenth-normal iodine solution and the excess of iodine is determined by titration with tenth-normal sodium thiosulphate. Two equivalents of iodine are required to oxidize one of formaldehyde. Put into a suitable flask a quantity of the sample estimated to contain fifteen milligrams of formaldehyde. In case the sample was one containing 35 to 40 percent of formaldehyde, this would correspond

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\*Journ. Am. Chem. Soc., 1903, 1031.

with 4 mls of a solution prepared by diluting 1 mil (accurately weighed) of the solution to the measure of 100 mls. Add 25 mls of tenth-normal iodine solution and sufficient of a solution of sodium hydroxide to bring the color of the mixture to a pale yellow. After 10 minutes add a small excess of hydrochloric acid, as shown by the change in color of the solution, and titrate the free iodine with tenth-normal sodium thiosulphate. Subtract the quantity in mls of thio-sulphate solution consumed from 25 and multiply by 0.001501 to find the weight in grammes of formaldehyde (HCHO) in the sample taken (one twenty-fifth part of the 1 mil originally weighed). The author points out that the method cannot be used if acetone or aldehydes, other than formaldehyde are present.

698. **Bernard H. Smith\*** modifies the method in some of its details, employing a stronger solution—up to five percent—for the test, which he finds especially suited to the determination of formaldehyde in weak solutions (e. g. 0.1 percent), and in absence of such impurities as other aldehydes. Smith employs a fifth-normal volumetric iodine solution, of which one mil consumed in the titration corresponds to 0.003002 gm. of HCHO.

699. Many other assay processes have been proposed, some of them having merit. Mention may be made of the sulphite methods respectively of Seyeneetz and Gibello† and of C. Kleber‡, the chlorinated lime method of Bräutigam\*\*, the gravimetric hexamethylene tetramine method§. C. Wallnitz has compared seven of the methods most commonly used, reaching the following conclusions: The hydrogen dioxidel and the iodine methods are superior to the others tested (these not, however, including the

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\*Zeitsch. anal. Chem., 1897, 18–24.

†Bull. Soc. Chim., 1901, 691–4; Chemical News 91,135 and 2365.

‡Pharm. Review, 1904, 22, 94.

\*\*Pharm. Zentr-h., 1910, 51, 915–6.

§Annual Report Conn. Expt. Station, 1899, 143.

||Deutsche Gerber Ztg., 1–4, 6, 8, 12; Zeitsch. f. ang. Chem., April 7, 1903.

cyanide method) when the reagents are freshly prepared; the ammonium chloride method of Schiff, as well as the Legler ammonium method lacks distinctness in the end-point reading, as does the aniline volumetric method (condemned also by B. H. Smith;) while the gravimetric silver nitrate method of L. Vanino is declared practically worthless.

700. Formaldehyde in tablets is best separated by dissolving the tablets in water and distilling in a current of steam. The distillation must be continued 2 hours to insure recovery of polymerized formaldehyde. The distillate is to be treated with iodine (697) or assayed by other approved method.

## FORMIC ACID

701. **Determination by oxidizing with potassium permanganate** in alkaline solution\*. Put into a stoppered pressure flask the sample containing not more than 0.2 gm. of formic acid dissolved in 20 mls of water. Add 50 mls of tenth-normal potassium permanganate and 0.5 gm. of anhydrous sodium carbonate. Stopper the flask securely and heat 30 minutes on the water bath, then cool, acidify with sulphuric acid, add 0.25 gm. of potassium iodide and after 2 minutes titrate the excess of iodine with tenth-normal sodium thiosulphate. Subtract the quantity of the latter in mls from 50 and multiply by 0.002301 to find the quantity of formic acid present. (It is perhaps better to conduct the oxidation at room temperature, allowing 2 hours for its completion.)

702. **Modified permanganate method of A. Fouchet†.** Put into a flask 40 mls of a 5 percent solution of sodium carbonate and 20 mls of an 0.5 percent solution of potassium permanganate, add 0.05 gm. of the sample, dissolved in 10 mls of water. Heat in the water bath three minutes, cool and add 20 mls of diluted sulphuric acid (500 mls of strong acid in a liter) and 50 mls of a solution containing

\*E. Rupp, in *Zeit. Anal. Chem.*, 1906, 687-92.

†*Bull. Soc. Chim.*, 1912, 325-8.

in 1 liter 20 gm. of ferrous ammonium sulphate and 30 gm. of sulphuric acid. Titrate the excess of the ferrous sulphate with the potassium permanganate solution. Run a blank simultaneously. Subtract the amount of the permanganate solution consumed in the test from that consumed in the blank, and multiply the difference in mls by 0.00351 to find the quantity of formic acid contained in the sample. For accurate results the exact titre of the permanganate solution should be ascertained and the factor should be deduced from this. Compare the factor in the foregoing paragraph. The theoretical figure would seem to be 0.00364 rather than 0.00351. (Ed.)

**703. Determination by reduction of mercuric to mercurous chloride.** H. Franzen and F Eggert† direct the following procedure. Dissolve the sample, which should not contain more than 0.25 gm. of formic acid, in 1 liter of water, add 50 mls of a reagent prepared by dissolving 200 gm. of mercuric chloride, 300 gm. of sodium acetate and 80 gm. of sodium chloride in water q. s. to make 1 liter. The reagent should be allowed to stand two days, and then decanted from a slight precipitate before use. Heat the mixture three hours on the water bath. Collect the precipitated mercurous chloride on a tared filter, wash it well with hot water, dry it at 100° C. 6 hours, then keep it in a vacuum over sulphuric acid or potassium hydroxide 15 hours and weigh. Each gm. of the precipitate corresponds with 0.097475 gm. of formic acid.

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†Journ. f. prakt. Chem., 1911, 323-5.

## GERANIOL (RHODINOL)

704. **The chief constituent of oil of rose** and of palma rosa oil (distilled from a species of grass), geraniol, occurs also as a component of many volatile oils, notably those of lemon grass, citronella, rose geranium, and in small quantities of orange flowers, ylang-ylang, etc. It is the lemonol of Barbier and Bouveault, and the rhodinol of Erdmann and Huth (not rhodinol of Barbier and Bouveault, which is citronellol), classed as a diolefinic alcohol having the formula  $C_{10}H_{18}O$  (isomeric with linalool) and converted by oxidation into citral ( $C_{10}H_{16}O$ ). It may be separated from other constituents of a volatile oil by treatment with calcium chloride, with which it forms a crystalline compound which is decomposed by water, otherwise it may be obtained in pure form by reaction with phthalic anhydride to form a crystallizable acid ester from which geraniol can be reconstituted by saponification with alcoholic potassium hydroxide. For assays see (536) to (538).

## GLYCEROPHOSPHATES

705. **The official assay processes** for these salts are on the whole disappointing in that they are not based on the proportion of glycerophosphoric acid potentially present in them. In the calcium salt it is calcium oxide which is determined, although there is a supplementary test by ignition, in which the residue obtained and weighed is calcium pyrophosphate. This, taken in connection with the requirement that the salt shall not respond at once in the cold to the ammonium molybdate test for a phosphate, while on heating it gives the familiar yellow precipitate, is sufficiently conclusive.

706. **In the case of the sodium salt**, however, the assay consists merely in an alkalimetric test. To make this convincing, it would be necessary to show the absence of other salts having an alkaline reaction

and of this we find not a word in the text. Ignition of the salt to a pyrophosphate would go far towards rendering the assay satisfactory. (One gm. of the official salt yields on ignition not less than 0.41864 gm. sodium pyrophosphate).

707. A **convincing assay** of a glycerophosphate would show, 1st the glyceryl content of the salt and 2nd the percent of phosphorus it contains. The first can be easily determined by the potassium dichromate method described in (155)\*. The second can be determined perhaps most easily by igniting a sample with sodium carbonate and potassium nitrate, dissolving the melt in nitric acid and precipitating the phosphoric acid with ammonium molybdate reagent.

708. An alternative method is proposed by M. Francois and E. Boismenu† as follows: Mix 0.5 gm. of the sample (of a glycerophosphate) in a 150 mil flask with a mixture of 10 gm. of sulphuric acid, 10 gm. of water and 4 gm. of potassium dichromate, added in 3 portions. The reaction is attended with evolution of carbon dioxide. Boil the mixture 2 hours under a reflux condenser, transfer to a half liter measuring flask and fill to the mark with water. Add to 50 mils of this solution 10 gm. of crystallized sodium sulphate followed by 20 gm. of sodium nitrate, and heat on a water bath until solution is complete. Add 300 mils of molybdic reagent and continue heating on the water bath 2 hours, after which the precipitate is to be collected and the determination of phosphoric acid made in the usual manner. (Official sodium glycerophosphate contains not less than 9.767 percent of phosphorus, corresponding with 35.041 percent of magnesium pyrophosphate. The official calcium glycerophosphate contains not less than 14.475 percent of phosphorus, corresponding with 51.93 percent of magnesium pyrophosphate.)

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\*Each mil of tenth-normal iodine solution corresponds with 0.16299 gm. of crystallized  $[2H_2O]$  or 0.15012 gm. of anhydrous calcium glycerophosphate; 0.2187 gm. of crystallized  $[5H_2O]$  or 0.15436 gm. of anhydrous sodium glycerphosphate; or 0.2221 gm. of crystallized  $[3H_2O]$  or 0.16586 gm. of anhydrous potassium glycerphosphate.

†Journ. Pharm. Chim., 1914 (7), 11, 65.

## HEXAMETHYLENAMINE (HEXAMETHYLENE-TETRAMINE)

**709. Hexamethylenamine is split by boiling** with an acid into ammonia (2 molecules) and formaldehyde (3 molecules). This renders determination of the compound a simple matter since either ammonia or formaldehyde can be determined with precision. W. A. Puckner and W. S. Hilpert\* employ the former method, proceeding as follows: Dissolve the sample estimated to contain 0.25 gm. of hexamethylenamine in 500 mls of hot water, add 10 gm. of potassium hydroxide and boil in an open flask half an hour to drive off any ammonia that may be present as an ammonium salt. Add 125 mls of diluted sulphuric acid and boil one and one half hours, by which ammonium sulphate is formed quantitatively. Cool the solution, add 50 mls of a 30 percent solution of potassium hydroxide and distil off two-thirds of the mixture, receiving the distillate in 100 mls of tenth-normal sulphuric acid. By residual titration the quantity of acid neutralized is found. Each mil of this corresponds with 0.0035035 gm. of hexamethylenamine.

**710. A simplified form of the above assay is** made official in the British Pharmacopoeia of 1914. Absence of any salt of ammonia in the sample is presupposed, and this is a requirement of the pharmacopoeias generally. The following is the procedure; Dissolve 1 gm. of the sample in 10 mls of water in a 100 mil beaker, add 35 mls of normal sulphuric acid and heat on a water bath until all odor of formaldehyde has been driven off, adding water if necessary to replace that lost by evaporation. Titrate the residual acid with normal sodium hydroxide, of which not more than 7 mls should be required for neutralization, methyl orange being used as indicator. Each mil of normal acid neutralized by the ammonia formed in the reaction corresponds with 0.0035035 gm. One gm. of

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\*Journ. Am. Chem. Soc., 1908, 1471-4.

pure hexamethylene-tetramine would correspond with 28.543 mls of normal sulphuric acid. The test requires therefore that the product shall contain not less than about 98 percent of hexamethylene-tetramine.

711. **W. O. Emery and C. D. Wright\*** adopt the second alternative [See (709)], determining the formaldehyde by the method of W. Stuewe†. The hydrolyzed hexamethylenamine is treated with a modified Nessler's reagent, causing reduction quantitatively of mercury; the acidified mixture is treated with volumetric iodine solution, which reoxidizes the mercury and finally the excess of iodine is titrated with volumetric thiosulphate, giving data for calculating the result. The procedure is as follows: In a suitable flask, fitted with a reflux condenser, boil 0.5 gm. of the sample 15 minutes with 100 mls of water and 25 mls of 10 percent hydrochloric acid. Cool, wash the condenser tube with a little water and transfer the mixture quantitatively to a 250 ml measuring flask, finally diluting to the mark with water. Of this solution 10 mls, representing 0.02 gm. of the sample, are pipetted into a 200 ml Erlenmeyer flask, containing a previously chilled mixture of 20 mls of a solution made by dissolving 10 gm. of mercuric chloride, 30 gm. of potassium iodide and 5 gm. of acacia in 200 mls of water, with 10 mls of a solution of 15 gm. of sodium hydroxide in 100 mls of water. Wash down the neck of the flask with a fine jet of water, rotate gently and let stand at least one minute. Add 10 mls of 40 percent acetic acid in such a manner that the neck of the flask is completely washed by the reagent, mix quickly and thoroughly by rotating and tilting the flask and immediately run in from a burette 20 mls of tenth-normal iodine solution and titrate the excess of iodine with twentieth-normal sodium thiosulphate, adding a few drops of starch solution near the end as indicator, the final color being a pale straw green. Subtract one-half the number of mls of thiosul-

\*Journ. Ind. and Eng. Chem., 1918, 606-8.

†Arch. Pharm., 1914, 430; Pharm. Ztg., 1914, 215.



phate used from 20 and multiply the remainder by 0.001167 to find the quantity in grammes of hexamethylenamine in the sample assayed.

**712. Determination by precipitation with mercuric chloride.** Fritz Schroter\* proposes to acidify the solution (the test particularly applicable to urine) with acetic acid and add a saturated solution of mercuric chloride. After half an hour, the precipitate is collected, washed with a little water, then transferred to a flask containing a saturated solution of sodium chloride. The mixture is heated on a water bath, the solution cooled and filtered and treated with potassium hydroxide to precipitate the mercury. Nitrogen is finally determined in the filtered solution by the Kjeldahl method. Each gm. of nitrogen corresponds with 2.5007 gm. of hexamethylenamine. A correction should be made on account of the solubility of the initial precipitate in water. The method however, is not expected to give more than approximate results. It is applicable particularly in case of a complex mixture in which determination of the hexamethylenamine cannot be made directly by the usual methods.

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\*Arch. Exp. Path. Pharm., 1911, (64), 161-6.

## HYDROCYANIC ACID

**713. Method of Lundell and Bridgman\*.** An ammoniacal solution of the cyanide is titrated with a standardized solution of nickel ammonium sulphate, using dimethylglyoxime as indicator. The reagents required are, (1) standard nickel solution (twentieth-normal), prepared by dissolving 12.25 gm. of nickel ammonium sulphate in water containing 2 mls of concentrated sulphuric acid, diluting to 1 liter with water and standardizing if necessary by determination of nickel, so that each mil shall contain 2.934 mg. of nickel; (2) dimethylglyoxime solution, prepared by dissolving 8.9 gm. of the reagent in 1 liter of 95 percent alcohol.

**714. The assay is conducted as follows:** Dissolve 0.5 gm. of the sample in a 250 mil Erlenmeyer flask in about 100 mls of water, add 1 mil of solution of ammonium hydroxide and 0.3 mil of the dimethylglyoxime solution and titrate with the standard nickel solution with vigorous shaking until a permanent red precipitate appears. Each mil of the standard nickel solution corresponds with hydrocyanic acid 5.404 mg. The authors claim for the method that it is accurate, free from ordinary interferences, rapid and particularly of value in the titration of certain double cyanides. The results are not affected by chlorides or ferrocyanides, but are too high in presence of ferricyanides or excess of caustic alkali.

**715. The Liebig assay†** is that most frequently used and gives results which are generally for practical purposes sufficiently exact. It is based on the fact that when a silver nitrate solution is added to a solution of a soluble cyanide no precipitate appears until one half of the cyanide is converted into silver cyanide. It is advantageous to add to the solution to be titrated a small quantity of potassium iodide. When the

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\*Journ. Ind. and Eng. Chem., 1914, 554-6.

†Annalen, 1851, 102.

sample to be tested contains free hydrocyanic acid, it is necessary to use precautions to prevent loss of this volatile compound. A solution of alkali hydroxide is put into a weighing bottle and the tare is taken, then the sample is introduced with minimum possible loss from volatilization and its exact weight is taken. The solution is placed in a beaker standing on a sheet of black glazed paper and diluted to 100 mls. Four or five mls of solution of potassium hydroxide are added, and titration is carried to the production of a faint permanent turbidity. Each ml of the volumetric solution corresponding with 0.005404 gm. of hydrocyanic acid.

716. **Several other methods** are in use, among them that of Vielhabert\*, in which the solution is made alkaline by the addition of magnesium oxide suspended in water, and titrated with tenth-normal silver nitrate, using potassium chromate as indicator. A modification of this method is adopted in U. S. P. IX for oil of bitter almond, conducted in the following manner: Dissolve 0.75 gm. of crystallized magnesium sulphate in 40 mls of distilled water, add 5 mls of half-normal sodium hydroxide and 2 drops of potassium chromate test solution and cautiously, just enough tenth-normal silver nitrate to give a permanent reddish tint. Mix with this by shaking about 1 gm. (accurately weighed) of the sample and immediately titrate with tenth-normal silver nitrate to the production of a permanent reddish tint. Each ml of the volumetric silver solution corresponds with 0.002702 gm. of hydrocyanic acid. The test, however, is fallacious and furthermore is of no practical value owing to the instability and the volatility of hydrocyanic acid. For determination of hydrocyanic acid in wild cherry bark see (525) to (527).

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\*Arch. Pharm., (3) 13, 408.

## iodoform

717. **Decomposition** of iodoform may be effected by **oxidation with nitric acid**. An assay may be made on this basis by the method proposed by Utz\* and found satisfactory by Gane and Webster†. Dissolve 0.5 gm. of the sample in 10 mls of a mixture of ether and alcohol (1 : 2). Add 50 mls of tenth-normal silver nitrate and then 1 ml of fuming nitric acid and heat on a water bath until the odor of nitrous acid has disappeared. Add 100 mls of water and titrate the excess of silver by Volhard's method. Subtract the volume in mls of the sulphocyanate solution used from 50 and multiply the remainder by 0.013126 to find the quantity of iodoform in the sample.

718. **An alternative method** (said to be "less practical") consists in decomposing the iodoform by prolonged boiling under a reflux condenser with a strong alcoholic solution of potassium hydroxide, neutralizing with nitric acid and determining the iodide formed by titration with silver nitrate.

## LACTIC ACID

719. **Lactic acid** as met with in commerce contains besides the acid itself a certain quantity of a compound which by hydration yields an additional amount of the acid. Hence the real strength (potential) of the acid is not revealed by a simple acidimetric titration as prescribed in U. S. P. VIII. The U. S. P. IX directs to add to the acid a measured excess of normal potassium hydroxide V. S., boil the mixture twenty minutes and then titrate the excess of alkali in the boiling solution with normal sulphuric acid V. S. According to E. Elvove‡, the application of heat in the assay process is not only unnecessary, but actually vitiates the result. He directs: Weigh

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\*Apoth. Zeitschr., 1903, 9/12.

†Zeitschr. Angew. Chem., 1909, 22, 1059-61 and 1190-1.

‡Am. Journ. Pharm., Jan. 1911, 14-19.

accurately about 2 gm. of the sample, add 50 mls of normal sodium hydroxide solution, mix well and let stand half an hour, then titrate the excess of alkali with normal sulphuric acid using phenolphthalein as indicator. Subtract the quantity in mls of volumetric acid used from 50 and multiply the remainder by 0.09005 to find the total quantity of potential and actual lactic acid in the sample taken for assay.

**720. Not very different** from the foregoing is Klapproth's method for determining both lactic acid and lactic anhydride in the commercial acid. One gm. of the acid is diluted with 20 mls of water and titrated with normal sodium hydroxide, using phenolphthalein as indicator. This shows the lactic acid present as such, plus one half of that represented by lactic anhydride. Three mls of normal sodium hydroxide are added, the solution heated 5 minutes on a water bath and the excess of alkali titrated with normal sulphuric acid; 1 mil of the latter is added in excess and the solution again heated 2 minutes on the water bath and the excess of acid finally titrated with normal sodium hydroxide. Subtract the volume in mls of the acid from that of the alkali used in the operations following the first titration and multiply by 0.1801 to find the lactic anhydride (in terms of lactic acid) in the sample examined. Half of this deducted from the lactic acid apparently shown in the first titration gives the lactic acid present as such in the sample.

## LECITHINS

**721. Lecithins are organic compounds** in which glycerophosphoric acid is united with oleic, palmitic or other fatty acid and with choline. They are contained in the seeds of many plants, and in other food stuffs, their importance depending on the fact that a lecithin enters largely into brain, nerve and embryonic tissues. While the value of commercial lecithin as a nutritive or as a therapeutic agent may be debatable, it has come into use by physicians under various forms, so that a standard of valuation has become a desideratum. Its separation from inorganic phosphates which are likely to accompany it is effected by extraction with ether or dehydrated alcohol, while its determination after such separation is readily made by fusion with an alkaline nitrate and an alkali, whereby the glycerophosphoric acid is converted into orthophosphoric acid which can be determined in the usual way.

**722. The details of the assay** as practiced by C. Virchow\* are as follows: Extract one gm. of the sample by boiling under a reflux condenser with three successive portions (10 mls) of dehydrated alcohol and filter through a filter of close texture into a tared flask. Wash the residue repeatedly with hot alcohol which is to be passed through the same filter to make up a total volume of 50 to 60 mls. Distil off the alcohol, weigh the residue and treat this with 14 mls of absolute ether. Pass the solution through an asbestos filter, wash thrice with absolute ether and distil off the solvent. Weigh the residue once more, treat this with 3 to 4 mls of strong nitric acid, transfer while hot to a platinum capsule and heat on the water bath until the acid is driven off. Fuse the residue with sodium hydroxide, dissolve the melt in hydrochloric acid, neutralize with ammonia and finally determine the phosphoric acid with magnesium mixture. One gm. magnesium pyrophosphate is taken to correspond with 6.975 gm. lecithin.

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\*Pharm. Zeit., 1911, 56, 724.

723. Since the lecithins vary greatly in molecular weight, it cannot be expected that results from this or any other prescribed assay will give results of any great degree of exactness. For example, the lecithin most commonly occurring in plants would have for its factor 6.265 instead of 6.975.

## MENTHOL

724. The pharmacopoeias generally give no method of determining the purity of menthol except by its physical characters and the absence of thymol or of more than a trace of non-volatile matter. Since the compound is readily acetylated however, an assay may be made by acetylation (132), saponifying the thoroughly washed and dried product with half-normal alcoholic potassium hydroxide and titrating the excess of alkali. Each mil of half-normal alkali consumed in saponifying the acetylated sample corresponds with 0.07808 gm. of pure menthol. See (569).

## METHYL SALICYLATE

725. The essential oils of *gaultheria* and of sweet birch consist almost wholly of the ester methyl salicylate, this being the official title (U. S. P. IX) of these oils as well as of the synthetic product. The purity of these oils may be demonstrated by their ready solubility in a solution of potassium hydroxide, even without application of heat, the characteristic odor of the oil being completely lost by conversion of the ester into potassium salicylate. The presence of even small quantities of almost any adulterant is shown by the separation of oily drops from the solution and the identity of the adulterant is generally revealed by its odor.

726. Assay of the oil may be made by treating the sample with an alkali hydroxide, washing the alkaline aqueous solution twice with chloroform to remove possible traces of volatile oils, fixed oils or hydrocarbons, adding excess of sulphuric acid to set

free the salicylic acid, shaking this out with chloroform or ether and finally determining its quantity by acidimetric titration (752). Each mil of a half-normal volumetric alkali corresponds with 0.07603 gm. of the pure ester.

**727. The U. S. P. official assay process** is content with saponifying the oil (2 mls, accurately weighed) by heating in a water bath two hours under a reflux condenser with 50 mls of half-normal alcoholic potassium hydroxide and titrating the excess of alkali with half-normal volumetric acid, using phenolphthalein as indicator. (Query: Why use *alcoholic* potassium hydroxide, and why allow so long a time for the saponification?)

**728. The iodine method** of Messinger and Vortman\*, modified by Kremers and Jamest. Saponify completely 1 gm. of the sample with 50 mls of normal potassium hydroxide and dilute the solution to 200 mls. Place 10 mls of this solution in a flask and heat to 60° C., add tenth-normal iodine solution until the mixture becomes permanently yellow, with formation, on shaking, of a dark red precipitate. Cool the solution, acidify it with diluted sulphuric acid and make up to 250 mls. Filter, rejecting the first 100 mls of filtrate. Titrate the excess of iodine in 100 mls of the filtrate with tenth-normal sodium thio-sulphate. Multiply the number of mls of the thio-sulphate solution by 2.5 and subtract the product from the number of mls of the iodine solution and multiply the remainder by 0.19968 to find the quantity of methyl salicylate in the aliquot taken (0.050 gm.).

**729. The generally accepted standard** for "natural" oils, such as that of sweet birch, commonly known as oil of wintergreen, is not less than 98 percent of methyl salicylate. There seems to be no ground for the preference given to natural oils over the synthetic.

**730. A flavoring extract of "wintergreen"** is assayed by Hortvet and West† in the following man-

\*Berichte, 22, p. 2321; 23, p. 2755.

†Pharm. Review., 16, p. 130; Gildemeister and Hoffmann's "Volatile Oils," Am. Ed. p. 335.

‡Journ. Ind. and Eng. Chem., 1909, 84.



ner, adopted tentatively by the A. O. A. C. Mix 10 mls of the sample with 10 mls of 10 percent solution of potassium hydroxide. Heat on the steam bath until the volume is reduced about one half, add a distinct excess of hydrochloric acid, sp. gr. 1.08, and extract with 3 portions (30 mls) of ether. Filter through a small dry filter into a tared dish, wash the filter with 10 mls of ether and allow filtrate and washings to evaporate spontaneously. Dry in a desiccator over sulphuric acid and weigh. Multiply the weight of the salicylic acid by 9.33 to find the percent by volume of methyl salicylate in the sample. Otherwise the acid may be determined by acidimetric titration with results almost equally precise.

### NITROGLYCERIN (GLYCERYL TRINITRATE)

731. **The only preparation of nitroglycerin** official in the U. S. P. IX is the spirit of glyceryl trinitrate, which is required to contain not less than 1.0 nor more than 1.1 percent by weight of the trinitrate. A ten percent range in strength in a preparation having such active properties seems liberal, but it is to be borne in mind that the solvent is very volatile so that greater strictness in requirements is not admissible. The official assay method is, however, very crude. A weighed quantity, not greatly exceeding 4 gm., is allowed to evaporate spontaneously (no limit of temperature specified), the residue dried in a desiccator over sulphuric acid and weighed. Absence of non-volatile impurities is taken for granted, but is not required by the text. The official method is capable of giving reasonably exact results as shown by the experiments of W. L. Scoville\*, provided no heat is used in the evaporation after the greater part of the alcohol has been driven off on a steam bath. The drying must be completed in a vacuum desiccator.

732. **Assay of Nitroglycerin** by use of the nitrometer has been practised by some, and is capable

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\*Am. Journ. Pharm., Aug. 1911, 362.

of yielding results of value, but only if strict attention is given to every detail of the procedure. There is not as yet available any exhaustive study of such details and consistent results therefore cannot be expected from this assay method.

**733. The Kjeldahl-Gunning method** has been successfully employed for this assay by Scoville†, who reports favorably also in the Dumas combustion method. In operating the former method, the alcohol must be first driven off completely (but probably under diminished atmospheric pressure), and the residual nitroglycerin must be dissolved in the acid before heat is applied. This is effected by continuous patient shaking.

**733½. The assay by saponification**, which was formerly official in the U.S.P., assumed that the only products of saponification with potassium hydroxide are potassium nitrite and glycerin, but it has been shown that such assumption was unwarranted and the method is wholly discredited.

**734. Assay of nitroglycerin tablets** is a more difficult problem, since the quantity of the drug present is very minute so that practically only colorimetric methods are available. The method most commonly employed is that of W. L. Scoville. Tablets representing 0.013 gm. ( $\frac{1}{8}$  grain) of nitroglycerin are crushed under ether in a cylinder by means of a glass rod, and extracted with successive portions of ether (about 15 mls each) until 100 mls of ethereal solution are obtained. Of this evaporate 5 mls (representing  $\frac{1}{100}$  grain of nitroglycerin) spontaneously in an evaporating dish. As soon as the solvent has disappeared, add 2 mls of phenoldisulphonic acid reagent, prepared by dissolving 25 gm. of pure white phenol in 150 mls of concentrated sulphuric acid, adding 75 mls of fuming sulphuric acid (13%  $\text{SO}_3$ ) and heating 2 hours at 100 C. Stir the mixture occasionally during 5 minutes, dilute with water to about 80 mls, add water of ammonia in slight excess, cool to room temperature and make up with water

†Ibid. p. 363.

to 100 mls. For colorimetric comparison use 1 mil of a standard solution of potassium nitrate, prepared by dissolving 0.8656 gm. of the pure fused salt in water sufficient to make 1 liter. One mil of this solution is the equivalent in nitrogen content of 0.01 grain (0.000648 gm.) of nitroglycerin. Evaporate this quantity to dryness and treat the residue as above with phenoldisulphonic acid and ammonia, and make colorimetric comparison of the two solutions in the usual manner.

**735. Modified Hay method\*.** This is a colorimetric method in which the nitroglycerin is saponified with an alcoholic solution of potassium hydroxide and comparison is made with a standard nitrite solution on the assumption that the saponification yields an exact definite quantity of potassium nitrite, which is improbable. The method is, however, used by some for approximate determination of minute quantities of glycerol trinitrate in tablets or pills.

## PHENOL

**736. For quantitative determination of phenol,** advantage is taken of the fact that it yields quantitatively with an excess of bromine the very insoluble compound, tribromophenol. This compound may be collected on a tared filter, washed, dried and weighed, but is more conveniently determined by treating it with potassium iodide from which, in an acid solution, it separates iodine in quantity equivalent to one half the bromine it contains. Titration of the iodine with standard thiosulphate solution then gives a measure of the amount of phenol present, each mil of the volumetric solution corresponding with 0.0015675 gm. of phenol. It is observed that after the solution has been decolorized by the thiosulphate solution, it frequently again develops color (starch having been used as indicator). L. de Konough recommends, to obviate this difficulty, that the liquid be decanted and any precipitate that may have been thrown down be dissolved in a little alcohol and the

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\*Journ. A. O. A. C., 1916, 362-3.

solution added to the decanted liquid. A 20 percent solution of potassium iodide and some starch paste are then added, and the titration carried out in the usual manner.

**737. Assay method of Redman and Rhodes\*.** Weigh accurately about 1.5 gm. of the phenol and dissolve in sufficient distilled water to make 1 liter. Transfer to a glass stoppered 500 mil bottle 20 mls of the solution, add 75 mls of distilled water and 5 mls of strong hydrochloric acid (sp. gr. 1.20). Add quickly tenth-normal bromine solution U. S. P. sufficient to give a yellow color to the solution and further one tenth of the quantity already added. Stopper the bottle at once and shake continuously one minute. Introduce quickly 5 mls of a solution of potassium iodide (1:5), avoiding as far as possible loss of bromine vapor. Insert the stopper and shake the bottle continuously three minutes. Rinse the stopper and neck of the bottle with distilled water and titrate the free iodine with tenth-normal sodium thiosulphate, adding at the last starch paste as indicator. Subtract the quantity of thiosulphate solution consumed from the total quantity of bromine solution, multiply the remainder by 0.0015675 to find the quantity of phenol in the sample taken.

**738. In the assay of the U. S. P. IX,** the prolonged shaking prescribed (30 minutes) is quite unnecessary, as shown by the test assays of Redman and Rhodes. The addition of chloroform before the final titration is also ill advised, rendering the titration needlessly tedious.

**739.** The method may be applied to solutions containing hexamethylene-tetramine. Formaldehyde however vitiates results materially, but it is possible to overcome this interference by adding to the solution before brominization strong water of ammonia, which reacts with the formaldehyde to produce non-interfering hexamethylene-tetramine. The ammonia (10 mls, 28 percent) is allowed to act five minutes and is then supersaturated by addition of 17 mls of hydrochloric acid (37 percent) before brominization.

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\*Journ. Ind. and Eng. Chem., 1912, 655-9.

**740. The method in modified form** may be employed for the determination of thymol and other phenolic bodies such as ortho-, meta- and para-cresol and likewise of mixtures of these latter with phenol. The modified procedure is as follows: Into a 500 mil glass stoppered bottle put 50 mls of water, 50 mls of sodium bicarbonate, approximately normal (84.1 gm. to the liter), and 15 mls of the phenolic solution, approximately tenth-normal in strength. Run in thirtieth-normal solution of iodine (iodine 4.23 gm.; potassium iodide 15 gm., to the liter) until the mixture remains a permanent brown iodine color (20 percent excess is recommended). Stopper the bottle securely and shake well for one minute, add then with due caution 60 mls of normal sulphuric acid, shake well and titrate the excess of iodine with tenth-normal sodium thiosulphate. See (771).

## PHENOLPHTHALEIN

**741.** No pharmacopoeial quantitative standards of purity have hitherto been fixed for phenolphthalein. Several assay methods, however, have been proposed, among which are the following:

**742. Assay by immiscible solvent.** Weigh accurately about 0.100 gm. of the sample, dissolve it in 10 mls of tenth-normal sodium hydroxide. Transfer the solution to a separator together with rinsings of the original container, shake out with two successive portions of chloroform (15 mls) to remove possible impurities, drawing off the chloroform into a second separator in which it is washed with a few mls of water which is to be added to the first separator. Render the phenolphthalein solution acid with diluted sulphuric acid, throwing out thus a copious white precipitate of phenolphthalein. Shake out with three or four successive portions of ether-chloroform (2 : 3); 25, 20, 20 and 15 mls, or q. s. Wash the ether-chloroform in separator No. 3 with water to which has been added a few drops of diluted sulphuric acid, evaporate off the solvent, dry the phenolphthalein at 100° C. to constant weight and weigh.

**743. Method by precipitation with iodine\*.**

Exhaust the powdered sample, which should contain 0.4 to 0.6 gm. of phenolphthalein, by percolation with an 8 percent aqueous solution of sodium hydroxide until the percolate is free from color. Add a solution of 2 gm. of iodine and 3 gm. of potassium iodide in 20 mls of water. The color of the solution changes first to blue and then to yellow. Add now strong hydrochloric acid to a decided acid reaction when the phenolphthalein is precipitated as tetraiodide. Collect the precipitate on a tared filter, wash it successively with water, alcohol and ether, dry it at 100° C. and weigh. Multiply the weight by 0.3869 to find the weight of the phenolphthalein contained in the sample.

**744. Volumetric method of V. Zotier†.**

Triturate a weighed quantity (0.5 to 1.0 gm.) of the powdered sample with sodium hydroxide free from carbonate, dissolved in a few mls of water, make up to 50 mls and filter. To 25 mls of the filtrate add diluted hydrochloric acid, until phenolphthalein begins to be precipitated and then add a drop or two of tenth-normal sodium hydroxide—sufficient just to redissolve the precipitate. Titrate with tenth-normal sulphuric acid to disappearance of red color, shaking vigorously after each addition of the reagent. Each ml of tenth-normal acid corresponds with 0.0318 gm. of phenolphthalein. Add as correction for water-solubility of phenolphthalein, 0.000184 gm. per ml of fluid titrated.

**745. Hydroxylamine assay of A. Mirkin‡.**

Dissolve in 40 mls of dehydrated alcohol 1 gm. of phenolphthalein, 0.8 gm. of hydroxylamine hydrochloride and 0.52 gm. of sodium hydroxide, in fine powders, and boil the mixture under a reflux condenser 2 or 3 hours until the liquid turns yellow. When cool, transfer to a 250 ml measuring flask, together with rinsings from original container, add 10 mls of 10 percent sulphuric acid and fill to the mark with water.

\*Kollo in Pharm. Praxis, 1903, No. 8.

†Bull. Soc. Chim. de France, 1910, (4) 7, 993.

‡Am. Journ. Pharm., 1914, 86, 307.

Neutralize 50 mls of the solution, representing 0.2 gm. of the sample, with tenth-normal sodium hydroxide, using methyl orange as indicator, then titrate with the same volumetric solution using phenolphthalein as indicator. Run a blank following exactly the foregoing routine with omission of the phenolphthalein excepting that used as indicator. Subtract the number of mls of tenth-normal alkali consumed in the blank from that in the assay and multiply the remainder by 0.159 to find the quantity of phenolphthalein contained in 1 gm. of the sample\*.

**745 $\frac{1}{2}$ . Tablets containing phenolphthalein** are to be treated with a solution of sodium hydroxide. The solution is to be made up to a definite volume, filtered and an aliquot is to be shaken out with chloroform to remove impurities, rendered acid with diluted sulphuric acid and the phenolphthalein recovered by shaking out with chloroform, following the details of (742).

## PICRIC ACID

**746. Determination of free picric acid** may be made, according to R. Feder†, by direct titration with volumetric alkali using phenolphthalein as indicator, if a sufficient quantity of the latter is used. The change of color from greenish blue to pure yellow at the end of the titration is quite sharp. A better plan is to add to the picric acid a mixture of potassium iodide and potassium iodate, and titrate the liberated iodine with sodium thiosulphate using starch solution as indicator (75). Each ml of tenth-normal thiosulphate solution (or of tenth-normal volumetric alkali, in the direct titration) corresponds with 0.0299 gm. of picric acid. The assay process of course assumes absence of other acids, in particular of mineral acids.

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\*The end point is not sharp. Perhaps methyl red would give a better result. G. C. Spencer of the Bureau of Chemistry says that the method is unsatisfactory because of uncertainty as to the end point when methyl orange is used.

†Zeitsch. Nahr, Genussm. 1906, 12, 216.

**747. To determine picric acid in picrates,** dissolve them in water, add hydrochloric acid in excess, extract the picric acid by shaking out with benzene, wash the benzene solution twice with water to remove hydrochloric acid, evaporate and proceed as in the foregoing paragraph. Residual picric acid, after precipitation of alkaloids by this reagent, may be shaken out and determined in the same manner.

**748. Assay by precipitation with nitron,** diphenyl-endo-anilo-dihydro-triazole ( $C_{20}H_6N_4 = 312.17$ ), which forms with picric acid, a compound practically insoluble in water. Max Busch and G. Blume\* direct to add to 150 mls of a solution containing 0.2 gm. of picric acid 1 to 2 mls of diluted sulphuric acid and heat the mixture just to boiling. Add 10 mls of a solution of 1 gm. of nitron in 10 mls of 5 percent acetic acid. When cold, collect the precipitate of nitron picrate in a Neubauer crucible, wash with 50 to 100 mls of cold water, dry one hour at  $110^\circ C.$  and weigh. Multiply the weight by 0.4232 to find the weight of the picric acid. Nitrates must be absent, also bromides, iodides, chlorides, chlorates, perchlorates, chromates and nitrites. A less direct method has been proposed by F. Utz† in which the picric acid is oxidized by means of hydrogen peroxide to nitric acid which is then determined by precipitation with nitron.

**749. Certain alkaloids,** notably cinchonine and berberine, precipitate picric acid very completely from solutions acidulated with dilute sulphuric (but not with hydrochloric) acid, and have been employed for quantitative determinations of the acid.

**750. Assay by precipitation with night-blue** (tetramethyl-tolyl-triamido-diphenyl-naphthyl-carbinolhydrochloride) or with crystal-violet (hexamethyl-rosoaniline hydrochloride) has been practiced by Kay and Appleyard‡ as volumetric processes. Dissolve

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\*Zeitschr angew. Chem., 1908, 21, 354-6.

†Zeitsch. anal. Chem., 1908, 47, 140-4.

‡Journ. Soc. Dyers, etc., IV, 83.



0.2118 gm. of purified night-blue in 100 mls of water and add from a burette a solution (1:1000) of the picric acid sample until the blue color is completely discharged. If the picric acid is 100 percent pure, it will require exactly 100 mls of the solution. Otherwise the purity percentage will be found by dividing 10,000 by the number of mls of the picric acid solution consumed. If crystal violet is used, the quantity to be taken is 0.1932 gm. instead of 0.2118 gm.

## RESORCINOL

751. **Determination of resorcinol** may be made with a good degree of exactness by use of a bromine solution after the general method for phenols of Schryner\*. The details of the method as applied by Degener† to resorcinol have been carefully studied by C. M. Pence‡ whose method, except in one particular has been made official in U. S. P. IX. Strict adherence to detail is important in the assay. According to Pence; dissolve in distilled water about 1.5 gm. of resorcinol, filter if necessary and make up to 500 mls. Transfer 25 mls of the solution to a glass stoppered bottle, add 50 mls of tenth-normal bromine solution and 50 mls of distilled water, followed by 5 mls of strong hydrochloric acid, shake and let stand one minute, dilute with 200 mls of water, add 5 mls of a 20 percent solution of potassium iodide, shake vigorously and let stand 5 minutes. Titrate the liberated iodine with tenth-normal sodium thiosulphate, using starch as indicator. Subtract the quantity of thiosulphate solution used in mls from 50 and multiply the remainder by 0.03668 to find the quantity in grammes of resorcinol present in the sample taken for assay.

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\*Journ. Soc. Chem. Ind., 1899, 558.

†Journ. prakt. Chemie, (2), 20, 322.

‡Journ. Ind. & Eng. Chem., Nov. 1911, 820-2.

## SALICYLIC ACID

**752. Determination of the free acid** is easily made by acidimetric titration. The U. S. P. IX directs to dry the acid to constant weight in a desiccator over sulphuric acid before weighing out a sample for assay. This is to be dissolved in diluted alcohol (strictly neutral) and titrated with tenth-normal barium hydroxide. Each mil of tenth-normal alkali corresponds with 0.013805 gm. of salicylic acid. The U. S. P. standard is not less than 99.3 percent of absolute acid. Lime water, standardized immediately before use on tenth-normal hydrochloric acid, can be conveniently substituted for the barium hydroxide.

**753. According to A. Seidell,\*** salicylic acid can be determined even in presence of benzoic acid in exactly the same manner as thymol, by the method described in (771). Each mil of tenth-normal sodium thiosulphate consumed in the final titration corresponds with 0.0069025 gm. of salicylic acid. In case of an alkali salicylate, it is evident that the alkalinity of the base must be taken into account, this being determined by ignition of the salt.

**754. Salicylates of alkaline bases** may be assayed by the U. S. P. general method (conversion into carbonate by ignition and alkalimetric titration of the residue) or by the alternative method described in (616). More convenient is the plan outlined in (639), which permits the determination of both base and acid in the same sample.

## SALOL

**755. Phenyl salicylate (salol) is easily hydrolyzed** by solutions of alkali hydroxides, with formation of sodium salicylate and sodium phenylate. Determination can then be made, (a) of the phenyl constituent (756); (b) of loss of alkalinity, due to conversion of sodium hydroxide into saline compounds; (c) of the salicylic acid formed as salicylate in the reaction (757).

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\*Am. Chem. Journ., 1912, 47, 508-26.

**756. Assay Process.** Weigh accurately about 0.9 gm. of salol in a 150 mil Erlenmeyer flask, add 50 mls of a solution of sodium hydroxide (2.5 percent) and heat five minutes on a boiling water bath. Transfer when cold to a 100 mil measuring flask, making up solution and rinsings to 100 mls. Measure 10 mls of this solution into a glass stoppered 500 mil flask, add 200 mls of water, followed by 30 mls of tenth-normal bromine solution and 5 mls of hydrochloric acid (U. S. P.), immediately stopper the flask and shake one minute, then at intervals during half an hour. Add 5 mls of a 20 percent solution of potassium iodide and at once stopper the flask again. Shake at intervals during 15 minutes, then titrate free iodine with tenth-normal sodium thiosulphate, using starch as indicator. Subtract the quantity in mls of thiosulphate solution used from 30 and multiply the remainder by 0.003568 to find the quantity of salol in the aliquot taken for the assay.

**757. Alternative process.** Weigh accurately 1 gm. of the sample in a 150 mil flask, add 50 mls of a solution of sodium hydroxide (2.5 percent) and heat on a boiling water bath five minutes. When cold transfer to a separator, add diluted sulphuric acid in excess and shake out the liberated salicylic acid with several successive portions of chloroform, wash the chloroform solutions with 10 mls of water, unite them in a flask, add 15 mls of water and a few drops of methyl red indicator\* and titrate the salicylic acid with tenth-normal alkali, each mil of which corresponds with 0.021408 gm. of salol.

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\*Titrate until after shaking well the aqueous stratum becomes yellow. Phenolphthalein may also be used as indicator.

## SODIUM CACODYLATE

758. **Pure sodium cacodylate** is neutral to phenolphthalein, but alkaline to methyl orange. The commercial salt contains free cacodylic acid. To determine the percent of total sodium cacodylate in a sample, in terms of the neutral salt, H. Imbert and A. Astruc\* direct to dissolve 1.6 gm. of the salt in 10 mls of water and neutralize exactly with sodium hydroxide, using phenolphthalein as indicator, then to add methyl orange indicator and titrate with tenth-normal hydrochloric or sulphuric acid. Each mil of tenth-normal acid consumed in the titration corresponds with 0.0160 gm. of anhydrous sodium cacodylate. The requirements of the U. S. P. IX is for the equivalent of not less than 72 nor more than 75 percent of the anhydrous salt. A pure salt containing 3 molecules of water of crystallization would show 74.75 percent of the anhydrous salt.

## TANNIC ACID

759. **The astringent principles** which exist in many plants and give to some their medicinal value are known as tannins. In chemical constitution they form a natural group, and certain reactions are common to all or nearly all, but they are of complex molecular structure and their characteristic reactions notoriously lack quantitative definiteness. Hence any standardization of astringent drugs must lack precision, and must as yet rest on an empirical basis. The group is sharply divided by the behavior of its members toward gelatin, those which form with this an insoluble compound constituting the tannins rightly so called, having the property of converting the skins of animals into leather, the other group consisting of astringent compounds such as gallic acid, which have not that property.

760. **Standard for official tannic acid.** There should certainly be prescribed in the pharmacopoeia a standard of some sort for tannic acid. The test

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\*Journ. Pharm., 1899, 392-5.

should be a simple one, requiring no special apparatus or reagents hard to procure. Ferric hydroxide suggests itself as a suitable reagent. It is easily prepared from solution of ferric sulphate or of ferric chloride, by precipitation with excess of sodium hydroxide, and thorough washing to remove soluble salts. A definite quantity of the iron solution should be used, and the ferric hydroxide prepared from it thoroughly washed and suspended in distilled water sufficient to make a prescribed volume. One gm. of the tannin should be dissolved in water, filtered and made up to 100 mls. An aliquot part of this solution should be mixed with the magma of ferric hydroxide, shaken well and allowed to stand a specified time, after which the mixture is to be filtered, an aliquot part of the filtrate evaporated, and the residue dried and weighed. The weight should not exceed a specified amount, representing a maximum of permissible impurity. A corresponding quantity of the tannin solution should be evaporated and the residue dried and weighed. The difference in weight between the two residues is the weight of tannic acid contained in the aliquot taken, and for this a minimum value should be prescribed. Alternative procedures might be based on absorption of tannin by hide powder, silk or catgut or other substitutes mentioned in (765) and (770) or else the copper method of assay of (762) may be adopted.

**761. Assay of tanning materials.** A detailed account of the practical assay processes in common use is not here in place. The Löwenthal method or some modification of it is widely employed. It is an oxidation process, by which different samples of the same tanning material may be compared with a close approximation to exactness, although in a comparison of different materials the conclusions reached may be very fallacious.

**762. Precipitation as a copper salt.** Copper acetate throws down from hot solutions of tannins precipitates of definite composition from which the relative proportion of the particular tannin can be

determined at least approximately. D. B. Dott\* directs to add to the warm infusion a hot solution of cupric acetate, to boil the mixture, collect the precipitate on a tared ashless filter, wash it with hot water, dry it at 110° C. to constant weight and weigh. Ignite the dried precipitate, dissolve the residue in a little nitric acid, evaporate, dry; ignite once more and weigh as cupric oxide. Multiply the weight of the cupric oxide by 0.8 as an approximate factor, and subtract the product from the weight of the dried precipitate of cupric tannate to find the weight of the tannin contained in the sample taken. Gallic acid is not precipitated, but some tannins like that of larch which are useful for tanning also yield no precipitate. The exact conditions under which the precipitation is made should be prescribed, and the behavior of the different tannins should be studied.

**763. Precipitation as a Zinc Compound** has been resorted to† (for determination of tannin in wine), the reagent employed being a solution of zinc acetate containing a large excess of ammonia. Gallic acid is not precipitated. To find approximately the weight of the tannin (anhydrous), weigh the dried precipitate, ignite it and subtract 80 percent of the weight of the zinc oxide, from the weight of the dried precipitate.

**764. Precipitation as ferric tannate.** This method should yield useful results. The ferric tannate may be ignited and the weight of tannin it represents can be calculated. Nickel also may be used as a precipitant, and for some tannins also antimony, but thus far no precipitation method has come into general acceptance.

**765. Assay by Hide Powder** or its equivalent. Hide powder absorbs from an aqueous solution any tannin it may contain. Assays of tanning material based on this principle are commonly practiced, but the details need not be given here. The quantity of

\*Journ. Soc. Chem. Ind., 1915, 1124; see also paper by Meyer in Chem. Ztg., XIV., 1202.

†A. Carpené, Journ. Chem. Soc., XXVIII, 1054.

tannin is determined either directly by the difference in extractive in the solution before and after treatment with the hide powder, or by inference (a) from reduction in the specific gravity or (b) from change of refractive index. As substitutes for hide powder, purified catgut,\* formalin-gelatin†, casein freed from fat‡ and silk freed from gum§, have been proposed. In the case of catgut, the determination is based on its increase in weight by absorption of tannin (the weight taken of course in the dry condition in all cases). These methods have value when applied with every attention to detail in the comparison of different samples of the same product.

**766. Iodometric assay.** Fairly good comparative results have been obtained by oxidation of tannin with iodine. Boudet|| prepares a solution containing in each liter 4 gm. of iodine and 8 gm. of potassium iodide. Each mil of this solution corresponds with 0.00455 gm. of gallo tannic acid or 0.00467 gm. of gallic acid. An aqueous solution is prepared each liter of which represents 4.55 gm. of the sample to be examined for tannin. To 10 mils of this solution, 10 mils of the iodine reagent are added and the mixture is set aside for 2 hours. The excess of iodine is then determined by titration with a standard solution (7.81 gm. to the liter) of sodium thiosulphate, corresponding in strength with the iodine solution. Subtract the quantity of thiosulphate solution expressed in mils from 10 and multiply by 10 to find the percent of tannin (in absence of gallic acid). Detannate a portion of the tannin solution with hide powder, and treat 10 mils of the detannated solution with iodine. Deduct the indicated tannin (which represents in fact gallic acid) from the first result. Care must be taken of course to avoid any oxidation of the tannin in the process of extraction or during the time the iodine is acting on the solution.

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\*A. Girard, in Journ. Soc. Chem. Ind. I, 464.

†Schmitz-Dumont, in Zeit. f. offent. Chem., III, 209.

‡M. Nierenstein, in Chem. Zeit., 1911, 35.

§L. Vignon in Compt. Rend., CXXVII, 289.

||Bull. Soc. Chim., 1906, 760-2.

**767. Assay by precipitation with strychnine.** S. R. Trotman and J. E. Hackford\* exhaust the material with alcohol in a soxhlet apparatus, concentrate to 50 mls, then dilute with water to 100 mls and filter. An aliquot of the filtrate (25 mls) is transferred to a 250 ml flask, 95 mls of water are added then a solution of 0.25 gm. of strychnine in 50 mls of alcohol, diluted with water to 100 mls, and finally water is added to make 250 mls. The precipitated strychnine tannate is collected on a Gooch filter, drier in a vacuum at 60° C. and weighed. Each gm. represents 0.491 gm. of tannin.

**768. Cinchonine is another alkaloid** which has been used in determination of tannin with fairly good results. If applied to tea, the caffeine must be first extracted by shaking out with chloroform. The composition of these precipitates must vary with different tannins, and probably with variations in the conditions.

**769. Gasometric determination of tannin.** W. Vaubel and O. Scheuer† propose a method depending on the quantity of oxygen absorbed from a mixture of the tannin solution with a certain quantity of sodium hydroxide. Under the conditions prescribed each gm. of oxygen absorbed corresponds with 3.049 gm. of tannin.

**770. Precipitation of tannin by activated aluminum.** H. Wislicenus‡ in 1905 suggested the use of "activated" aluminium as a substitute for hide powder for the absorption of tannin from its solutions. E. Kohn Abrest§ gives details of the assay method as follows: Activate a piece of aluminium foil about 1 mm. in thickness and weighing 3 gm., by immersing it for 3 minutes in a 3 percent solution of mercuric chloride. Place this in the tannin solution (100 mls) and let it remain there 4 hours. If at the end of that time the solution still shows presence of tannin by the ferric chloride test, use a second piece

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\*Zeit. anal. Chem., 1905, 44, 96-106.

†Zeitsch. angew. Chem., 1906, 2130-3.

‡Ann. Chim. Anal., 1913, 349-51.



of aluminium. When free from tannin, the solution is to be filtered and the quantity of extractive it contains is to be determined. The extractive in the original solution having been already determined, the difference represents the amount of tannin present. A little aluminum is dissolved. For this make allowance by incinerating the residues and ascertaining the difference in weight of ash, to be deducted from the above result.

## THYMOL

**771. Determination of thymol** has been repeatedly attempted by modifications of the method of Koppeschaar for phenol, but with results not wholly satisfactory. A plan has been found, however, by A. Seidell\* by which exact and consistent results are believed to be assured. Weigh accurately in a 300 mil glass-stoppered bottle about 0.2 gm. of the sample, dissolve in 1 mil of carbon tetrachloride and add 100 mls of water. Pour over the mixture bromine vapor until the color, after thorough shaking, indicates that bromine is present in decided excess. After half an hour, add 5 mls of carbon disulphide and 5 mls of a 20 percent solution of potassium iodide. The iodide set free by the action of the excess of bromine on the potassium salt is titrated with tenth-normal sodium thiosulphate. Add 1 mil more of the potassium iodide solution, and if this causes further liberation of iodine, titrate this also. Then add 5 mls of a 2 percent solution of potassium iodate, by which iodine corresponding in amount to the quantity of bromine which has combined with the thymol is set free. This is to be titrated with tenth-normal sodium thiosulphate, the titration being continued with thorough shaking of the mixture to complete disappearance of iodine color. Each equivalent of iodine corresponds with one equivalent of bromine in form of hydrobromic acid, so that one mil of the thiosulphate solution represents 0.0075055 gm. of thymol.

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\*Am. Chem. Journ., 1912, 47, 508-26.

**772. Determination of thymol in an essential oil,** e. g. oil of thyme. Kremers and Schreiner\* adopt a modification of the iodine method of Messinger and Vortmann†. Weigh 5 mls of the oil and transfer it by aid of 5 mls of petroleum benzin to a burette having a glass stopcock, add 5 mls of a 5 per cent solution of sodium hydroxide, shake the fluids together and allow them to separate. Draw off the aqueous solution into a 100 mil measuring flask. Repeat the extraction with successive portions (5 mls) of the soda solution as long as there is diminution in volume of the oil-solution. Make up the volume of the alkaline solution to 100 mls, transfer 10 mls of it to a 500 mil measuring flask, add tenth-normal iodine solution until a few drops of the mixture show a brown color and no milkiness when acidulated with hydrochloric acid. Acidulate the solution and dilute to 500 mls. Titrate 100 mls of this with tenth-normal sodium thiosulphate. Subtract five times the quantity in mls of thiosulphate solution consumed from the total amount of volumetric iodine and multiply the remainder by 0.03753 to find the quantity of thymol in the sample of oil taken.‡.

**773. Carvacrol, the valuable constituent of Trieste and Smyrna Oil of Origanum,** may be determined by a modification of the foregoing assay process. The iodine compound remains suspended, forming a milky mixture so that it is necessary to shake the mixture thoroughly and filter it before adding hydrochloric acid. The calculation is the same.

\*Pharm. Review, 14, p. 221; Gildemeister and Hoffmann's Volatile Oils (Kremers), p. 627.

†Berichte, 23, p. 2753.

‡The reaction is  $C_{10}H_{14}O + 4I + 2NaOH = C_{10}H_{12}OI_2 + 2NaI + 2H_2O$ .

**THYMOL IODIDE**

**774. Reduction by zinc dust** and determination of the iodine by silver nitrate, form the basis of the method proposed by Elvove\*. Introduce into a 500 mil Erlenmeyer flask about 0.25 gm., accurately weighed, of the sample. Add 10 mls of ether, 20 mls of half-normal alcoholic sodium hydroxide and 2 gm. of zinc dust, and boil one hour under a reflux condenser, then disconnect the condenser, add 10 mls of glacial acetic acid and again boil one hour under the reflux condenser. Rinse the condenser with about 10 mls of water into the flask, filter the solution and wash the residue with about 30 mls of hot water. To filtrate and washings, add 15 mls of tenth-normal silver nitrate and boil actively ten minutes, add 50 mls of diluted nitric acid (10 percent) and boil 5 minutes. Determine excess of silver in the filtered and cooled solution by titration with tenth-normal sodium thiocyanate, using 5 mls of 10 percent solution of ferric alum as indicator. Each mil of silver nitrate solution consumed corresponds with 0.0275015 gm. thymol iodide.

**775. In the method of Gane and Webster†** the thymol iodide is decomposed by ignition with an alkaline salt, and the iodide determined by titration with silver nitrate. Mix 1 gm. of the sample with 1 gm. of potassium and sodium tartrate and 5 gm. of dried sodium carbonate, place the mixture in a deep porcelain crucible and cover it with 2 gm. of a mixture (1 : 5) of potassium and sodium tartrate and dried sodium carbonate. Cover the crucible and heat gradually to fusion and maintain the heat for 45 minutes. Extract the residue with water and carefully neutralize an aliquot portion of it with diluted nitric acid (5 percent) using methyl-orange test paper as indicator. Insure exact neutrality by adding about 0.1 gm. of calcium carbonate, and titrate with tenth-normal silver nitrate, using potassium chromate as indicator. Acidify the liquid with nitric acid and

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\*Am. Journ. Pharm., Sept. 1910, 403-9.

†Zeitschr. angew. Chem., 1909, 22, 1190-1.

collect and weigh the silver iodide. This must correspond with the result of the titration, each mil of tenth-normal silver solution corresponding with 0.0275 gm. of thymol iodide. If there is discrepancy due to presence of a chloride find the true amount of iodide by

aid of the formula:  $x = \frac{23.48A - W}{91.46}$  in which A = num-

ber of mils of silver solution used in the titration, W, the weight of the precipitate and  $x$  the number of mils of silver solution corresponding with the iodide present

**776. The assay method of the U. S. P. IX** is a modification of the foregoing, the correction for possible presence of chloride being avoided by the expedient of converting the iodide (possibly mixed with chloride) into iodate by oxidation with potassium permanganate the iodate being then determined by the amount of iodine set free by reaction in acid solution with an excess of potassium iodide. The requirement of the U. S. P. is a minimum of 43 percent of iodine, the theoretical quantity in pure  $C_{20}H_{24}O_2I_2$  being 46.15 percent.

## Chapter IX

### Carbohydrates

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#### STARCH

**777. The purity of starch** is easily demonstrated by a microscopical examination. This, indeed, is the only way in which admixture with arrow root or corn starch of cheaper varieties of starch can be detected. All starches are more or less hygroscopic. In some cases a limit of hygroscopic moisture is fixed, e. g. in the German Pharmacopoeia wheat starch is not permitted to contain more than 12 percent. An ash limit of 0.5 percent is fixed for corn starch in the U. S. P. IX, which is wholly reasonable.

**778. Determination by Polarization** of the hydrolyzed starch solution\*. Mix 2.5 gm. of the finely powdered material with 10 mls of water, added gradually, add 20 mls of hydrochloric acid, sp. gr. 1.19; after 30 minutes rinse the thick solution into a 100 ml measuring flask with hydrochloric acid, sp. gr. 1.125, add 5 mls of 4 percent phosphotungstic acid solution and sufficient hydrochloric acid, sp. gr. 1.125, to make up 100 mls. Filter the solution and examine in a 200 mm. tube with a polariscope. Average for the common varieties of starch  $[\alpha] \frac{20}{D} 202$ . Results are stated to be 4 to 6 percent lower than by other methods. A similar method is that of Erich Ewers,† who states that when mixtures of wheat flour with water are allowed to stand 24 hours at room temperature there is a notable loss of starch by enzyme action. Maize starch undergoes a similar loss, but rice starch is scarcely affected.

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\*Carl J. Lintner, Zeitsch. Nahr. Genussm., 1907, 14, 205-8.

†Zeitsch. Offentl. Chem., 1908, 14, 150-7.

**779. Chemical determination of starch** is effected by converting it into dextrose and titrating with a volumetric copper solution. Mix 1 gm. of the sample with 10 mls of water, add 100 mls of boiling water, then 10 mls of hydrochloric acid (sp. gr. 1.125) and heat the mixture on a steam bath 2.5 hours in a flask fitted with a reflux condenser. Cool, render nearly but not quite neutral with sodium hydroxide and make up to 250 mls with water. Determine dextrose in an aliquot portion (50 mls) of the solution by Fehling's reagent as in (788) or preferably (793). Multiply the result by 0.93\* to find the weight of starch in the aliquot taken, and calculate result to a percentage basis. A second sample of the starch should be dried 2 hours at 110° C. to determine hygroscopic moisture, and the percentage of starch should finally be reported on the basis of the dried starch.

**780.** The following is an alternative method of converting starch into glucose. Prepare an infusion of malt by macerating 10 gm. of fresh finely ground malt for 2.5 hours at room temperature in 200 mls of water and filter. Determine the amount of cuprous oxide thrown down from Fehling's solution by 40 mls of the infusion. Mix 1 gm. of the sample with 10 mls of water, add 50 mls of boiling water and heat on the water bath 15 minutes. Cool to 55° C., add 20 mls of the malt infusion and maintain the mixture 1 hour at 55°. Heat again to boiling for a few minutes, cool to 55° C., add 20 mls more of the malt infusion and again maintain the temperature 1 hour at 55°. Cool, make up to 250 mls and filter, mix 200 mls of the filtrate with 20 mls of hydrochloric acid, sp. gr. 1.125, and heat in a boiling water bath, under a reflux condenser 2½ hours. Cool the solution, render it nearly but not quite neutral, make up to a definite volume with water. Determine dextrose by reduction of Fehling's solution as in (793), deducting the cuprous oxide thrown down by the infusion of malt.

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\*The theoretical factor is 0.90; the figure given in the text is better in accord with fact.

**781 ABBREVIATED TABLE**

for determining starch from precipitated  $\text{Cu}_2\text{O}$ . 100 parts d. glucose=93 parts starch.

Weight of Precipitated $\text{Cu}_2\text{O}$ (mg.)	Corresponding Weight of Dry Starch	Weight of Dry Starch Corresponding to Dif. of 1 mg. in Wt. of $\text{Cu}_2\text{O}$ .
00	0.0	0.450
20	9.0	0.415
40	17.3	0.415
60	25.6	0.410
80	33.8	0.420
100	42.2	0.415
120	50.5	0.420
140	58.9	0.420
160	67.4	0.430
180	75.9	0.430
200	84.5	0.435
220	93.2	0.435
240	101.9	0.440
260	110.6	0.445
280	119.6	0.450
300	128.6	0.450
320	137.6	0.450
340	146.6	0.455
360	155.7	0.460
380	164.9	0.465
400	174.2	0.470
420	185.6	0.475
440	193.1	0.480
460	202.7	0.485
480	212.4	0.485
500	222.1	.....

Example. Yield of cuprous oxide 443 mg. Find in table nearest number lower than this, which is 440, corresponding with 193.1 mg. starch. Difference =  $443 - 440 = 3$  mg. and  $3 \times 0.480 = 1.44$ . Finally  $193.1 + 1.44 = 194.54$ . If 200 mg. of the sample were taken for the test, the percent of starch is  $194.5 \div 2 = 97.3$

**782. Determination by the Refractometer\*.** Diastase is prepared by precipitating an aqueous infusion of a malt rich in protein with ammonium

\*L. M. Ljalin, Jn. Russ. Phys. Chem. Soc., 1909, 41, 472-6.

sulphate. Mix in a small mortar about 2 gm. accurately weighed of the sample in fine powder with 20 mls of water, gradually added, transfer to a 100 ml measuring flask with 60 mls more of water, add 0.2 ml of a 1 percent diastase solution, heat five minutes in a boiling water bath, cool to 55° C., add 0.25 mls more of the diastase solution, keep the temperature at 55° C. half an hour, cool and make up to 100 mls (at 17.5° C.) shake well, filter through a dry filter and examine at 17.5° C., in the Zeiss immersion refractometer. Make a blank using the same quantity of the substance treated with cold water, made up to the same volume and shaken repeatedly during half an hour. Subtract the reading given by this solution from the former reading and reckon 0.25 gm. of starch for each unit of the remainder.

**783. Precipitation of starch by iodine\*.** Heat a weighed sample of the material estimated to contain 0.1 gm. of starch with 5 mls of glycerin to a temperature of 190° C., for 5 minutes, dilute with water to 50 mls and filter. To an aliquot portion of the filtrate add a concentrated aqueous solution of iodine and potassium iodide, collect the precipitate of iodized starch on a filter, wash with boiling 90 percent alcohol and rinse into a platinum capsule with a jet of boiling water. Boil to expel iodine, evaporate, dry and weigh as starch. Ignite and deduct weight of ash if any.

**784. Oxidation of starch** by alkaline solution of **potassium permanganate**, by which the carbohydrate is converted into oxalic acid. Water-soluble substances are extracted from the sample, previously treated with ether. The process is the same as for determining glycerin by potassium permanganate, (164) but details of the assay have not been worked out.

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\*Lester Reed, in Chemical News, 1911, 104, 271.



## SUGAR

**785. Accurate determination of sucrose** may be most easily made by use of the polariscope, and this method is universally practiced in the commercial valuation of raw sugars. The instrument required, is, however, not part of the equipment of the average chemical laboratory. The requirement of the U. S. P. IX is that the specific rotation of sugar, determined at 20° C. in a solution in distilled water containing in 100 mls the equivalent of 26 gm. of sugar, previously dried to constant weight at 105° C., and using a 200 mm. tube, is not less than + 65.9°.

**786. Determination volumetrically** by a standard copper solution—Soxhlet's modification of Fehling's solution. See U. S. P. IX p. 557, but note that in Soxhlet's solution the quantity of copper sulphate is 34.639 gm. while in the U. S. P. formula it is 34.66 gm. For accurate work the solution must be standardized on pure sucrose, which can be procured from the Bureau of Standards, or can be prepared by repeated crystallization (with aid of alcohol) from the purest obtainable sugar. Dissolve 4.75 gm. of the pure sucrose in 75 mls of water, invert by method described in the following paragraph, neutralize exactly with sodium hydroxide and dilute to 1000 mls. Ten mls of this solution (containing 0.05 gm. of invert sugar) should reduce exactly 10 mls of the reagent.

**787. Method of inverting sugar for the reduction test.** Dissolve 4.75 gm. of the sample in 75 mls of distilled water, add slowly 5 mls of hydrochloric acid, sp. gr. 1.188, rotating the flask constantly. Immerse the flask in a water bath which is maintained at 70° C. The temperature of the solution should reach 69° in 3 minutes. Maintain that temperature as nearly as possible 7 minutes. Cool the solution to room temperature, neutralize exactly with sodium hydroxide, and dilute with distilled water to 1 liter.

**788. Routine of the volumetric test.** Place in a 400 mil beaker of alkali-resisting glass 25 mls each of the copper sulphate and alkaline tartrate solutions, add 50 mls of the inverted sugar solution,

(787) heat rapidly to boiling and boil exactly 2 minutes keeping the beaker covered all the time with a watch glass. Filter a portion of the solution without delay and test for residual copper by adding dilute acetic acid in excess and a dilute solution of potassium ferrocyanide. If copper is shown to be present, make a new experiment, using 52 mils of the sugar solution. Repeat the tests until 2 successive amounts are found which differ by 0.1 mil, one giving complete reduction, the other showing a trace of residual copper. The mean of these two may be taken as the exact quantity of sugar solution which will reduce the copper completely. The quantity of reagent used, under the conditions of the test, will require for complete reduction 0.247 gm. of invert sugar, corresponding with 0.235 gm. of sucrose.

**789. A close approximation** of the quantity of sugar solution that will be required, may be reached by the following expedient. Add to the mixture in the first test about 1 gm. of calcium carbonate. After boiling the mixture 2 minutes, (most conveniently in a small Erlenmeyer flask) remove the flame, when the solution will clear itself promptly, any residual copper being shown by the blue color of the solution. In the first test the color will be quite distinct. Add now successive portions of 0.5 mil, of the inverted sugar solution boiling 2 minutes after each addition, until a colorless solution remains after subsidence of the precipitate. The requisite quantity of sugar solution will now be approximately known, and only one or two additional experiments will be required to ascertain the exact quantity. Instead of depending on the color of the solution, one may remove a few drops of the clear fluid with a pipette and test for copper with acetic acid and potassium ferrocyanide. In any case however, the final tests are to be made *de novo* for exact results.

**790. Commercial sugar always contains traces of invert sugar**, so that results reached by the above method will always be a little high. The correction can be best ascertained by a gravimetric

determination, which may be made by the method prescribed in U. S. P. IX. The standard of purity of a well crystallized dry sugar is indeed best fixed by limitation of the quantity of invert sugar present, the official (U. S. P.) requirement being that the invert sugar shall not exceed 0.5 percent of the whole.

791. For practical purposes, the assay method of Herzfeld, determining (in the sample dried at  $105^{\circ}\text{C}$ . to constant weight) the percent of invert sugar, is generally all that is required. Determine moisture in the sample, then dissolve a quantity equivalent to 20 gm. of the dry substance in distilled water sufficient to make 100 mls. Filter if necessary. To 50 mls of the solution contained in a 250 ml beaker of alkali-resisting glass, (covered with a watch glass) add 50 mls of the alkaline cupric tartrate standard solution, apply heat so as to bring the liquid to boiling in approximately 4 minutes, then boil 2 minutes. Add 100 mls of recently boiled distilled water, filter at once through asbestos in a Gooch crucible, with suction. Wash the precipitate of cuprous oxide well with recently boiled water, then with 10 mls of alcohol and finally with 10 mls of ether. Dry it at  $100^{\circ}\text{C}$ . and weigh. Herzfeld gives a table for finding, from the weight in milligrams of copper corresponding with the cuprous oxide precipitated, the percent of invert sugar present. Substantially identical results are reached by following this **rule**: From the weight in gms of the cuprous oxide deduct 0.052 gm. and multiply the remainder by 4.918 for percent of invert sugar. This rule holds up to 1.5 percent.

792. **Gravimetric determination of sucrose.** The quantity of any reducing sugar is proportioned to the quantity of cuprous oxide formed under specified conditions in the test with Fehling's solution (not however in a strictly simple ratio). The sucrose must be inverted by the procedure described in (787). Three experiments are then to be carried out simultaneously. By the first, the total reducing action of the inverted sugar is ascertained, the second is a blank to determine how much cuprous oxide is thrown down by heating the reagents by themselves. In the third

test the uninverted sugar is used to ascertain how much of the cuprous oxide formed in the main test is due to the presence of inverted sugar in the sample. In this test use ten grams of the sugar instead of the smaller quantity used in the main test, calculating from the result the correction that would correspond with the smaller quantity.

**793. Routine of gravimetric reduction test\*.** Transfer 25 mls each of the copper and alkaline tartrate solutions to a 400 mil beaker of alkali-resisting glass and add 20 mls of the inverted sugar solution, representing 0.090 gm. of the sugar sample, or about 0.100 gm. of invert sugar. Add also 30 mls of distilled water. Cover the beaker with a glass watch and heat on an asbestos gauze over a Bunsen burner so regulated that boiling begins in 4 minutes. Boil the mixture exactly 2 minutes. Without diluting, filter the cuprous oxide on an asbestos felt in a porcelain Gooch crucible, using suction. Wash the cuprous oxide thoroughly with water at a temperature of about 60° C., then with 10 mls of alcohol and finally with 10 mls of ether, dry 30 minutes in a water oven at 100° C., cool in a desiccator and weigh as cuprous oxide. Correct the weight by deducting the cuprous oxide from the reagents used and that from invert sugar originally present in the sample. By aid of condensed table (page 369) one may now ascertain the percent of sucrose in the sample†. For comprehensive tables consult the original paper. See also Journ. A. O. A. C., 1916, 23-41.

**794. The authors give the following directions** for preparing the asbestos filter. Digest the asbestos, which should be of the amphibole variety, 2 or 3 days with dilute hydrochloric acid (1:3), wash free from acid and digest for a similar period with soda solution (strength not stated, presumably 5 percent), after which treat for a few hours with hot alkaline copper

\*L. S. Munson and P. H. Walker, Journ. Am. Chem. Soc. 1906, 663-686.

†Determine moisture in the sample by drying to constant weight at 105°, and calculate result to an anhydrous basis.

tartrate solution of the strength employed in sugar determinations. Wash the asbestos free from alkali, digest several hours with nitric acid, (strength not stated), wash free from acid and shake up with water for use. Load the Gooch crucible with a layer of the asbestos one-fourth inch thick. Wash this thoroughly with water to remove fine particles of asbestos, finally wash with alcohol and ether, dry 30 minutes at 100° C. cool in a desiccator and weigh. Use the same felt repeatedly, having dissolved the cuprous oxide in dilute nitric acid and then washed, dried and weighed the crucible as above.

**795. Most recent improvement in volumetric determination of reducing sugars.** The problem of the accurate determination **by titration** of reduced copper in the mixture remaining after completion of reduction, as a measure of the quantity of sugar in a given sample, seems to have been satisfactorily solved by W. B. Clark\*. The standard copper solution, a modification of that of Benedict, consists of:

Copper Sulphate, cryst.....	16 gm.
Sodium Citrate, cryst.....	150 gm.
Sodium Carbonate, anhydrous.....	130 gm.
Sodium Hydrogen Carbonate, anhyd....	10 gm.
Water to make.....	1 liter

Dissolve the copper sulphate in 125 mls of water, the remaining salts, separately, by aid of heat, in 650 mls of water. Pour the former solution slowly into the latter, with stirring, make up nearly to volume, cool, add water to make one liter and filter.

**796. The reduction is carried out** in flasks uniform in weight as well as in capacity and having as nearly as possible the same heat conducting values, the source of heat being also carefully regulated so as to insure uniformity of conditions in successive tests. The solution to be tested must be as nearly as possible neutral in reaction. Measure into the reducing flask 10 mls of this solution, representing not more than 0.030 gm. of reducing sugar, add 20 mls of the copper solution, close the flask with a two or three-hole

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†Journ. Am. Chem. Soc., 1918, 1759-72.

stopper through one of the holes of which passes a thistle tube, having the tip of the stem drawn down so as to deliver 50 mils of water in from 25 to 30 seconds. The heat is so regulated that boiling of the mixture will begin in 3 to 4 minutes, and the boiling is to be continued just 2 minutes.

**797. Determination of the precipitated cuprous oxide.** Remove from the source of heat, and add through the thistle tube 5 drops of hydrochloric acid sp. gr. 1.19, providing thus in the reduction flask an atmosphere of carbon dioxide. Cool the flask rapidly to room temperature, add little by little through the funnel tube about 4.5 mils of hydrochloric acid (sp. gr. 1.19), or just enough to produce a clear solution, faintly acid to litmus. Now add through the funnel tube 30 mils of distilled water. The apparatus must be so adjusted that at this point the tip of the funnel tube dips into the solution in the flask to a depth of 1 to 3 mm. Add 10 mils of tenth-normal iodine solution (or an equivalent quantity of twentieth- or twenty fifth-normal iodine solution), taking care that no air is swept into the flask with it. Rinse the funnel with 30 mils of distilled water. The iodine will generally at first throw down a precipitate of cuprous iodide, which will be later redissolved if the quantity of iodine added has been sufficient; otherwise more must be added, in measured amount. Volumetric sodium thiosulphate (preferably twenty-fifth normal) is then added, at first 1.5 to 2 mils at a time, until the color of the solution is a light emerald green, then starch solution is added and the titration is concluded in the usual manner. A blank test is carried through in which distilled water replaces the sugar solution. The difference in thiosulphate solution consumed is equivalent to the iodine consumed in reoxidizing the reduced copper, each mil of twenty-fifth normal thiosulphate corresponding with 0.0028628 gm. of cuprous oxide.

**798. The actual reducing power** of solutions containing a known quantity of the variety of sugar in question, is determined experimentally for the

conditions under which the test is made, so that it is not necessary to consult tables. The original paper of Mr. Clark should be carefully studied by any one who has occasion to make determinations of reducing sugars. The author shows incidentally that sucrose except in very large amounts has practically no reducing action when this method of testing is adopted. Alcohol does not appreciably affect results. Formaldehyde produces sufficient reduction to vitiate tests where the quantity of sugar is small.

## LACTOSE

799. **The purity of milk sugar** may be established by means of its several physical constants, particularly its action on the polarized ray. Its specific rotation, determined at  $25^{\circ}$ , in a solution in distilled water containing in 100 mls the equivalent of 10 gm. and using a 200 mm. tube, is from  $+52.2^{\circ}$  to  $+52.3^{\circ}$ .

801. **Determination of lactose** is commonly made by the copper reduction test, which is carried out exactly as prescribed in (793), the solution being made by dissolving 5 gm. of the dried sample in 200 mls of distilled water, filtering the solution and making up filtrate and washings to 1 liter. Fifty mls of this solution, corresponding with 0.250 gm. of the sample, should yield, if 100 percent pure, 0.3613 gm. of cuprous oxide. If the standard were made not less than 98 percent of crystallized lactose in the sample dried at  $110^{\circ}$  to constant weight, the precipitate of cuprous oxide from 0.250 gm. of the dried milk sugar must weigh not less than 354.3 mg. See p. 369 for table showing percentages of lactose corresponding with different quantities of cuprous oxide formed in the reduction test.

## GLUCOSE

802. **The glucose of the U. S. P. IX** is a concentrated solution in water of the products of hydrolysis of starch, viz: dextrose (d-glucose) and dextrans. It is not practicable to fix an exact standard of dex-

trose content. A limit might be arbitrarily established, determination of the dextrose being made after inversion of dextrin as in the starch assay, (778) and (779). Probably 98 percent of the dry substance would be a reasonable minimum. A maximum of 20 percent for water might be adopted, as in the British Pharmacopoeia. A limit should be set, as in U. S. P. IX, to the percentage of sulphur dioxide (or sulphites) and of arsenic. See following page for Table showing percentages of d-glucose corresponding with different quantities of cuprous oxide formed in the reduction test.



**802. Condensed table for determining sugars by Fehling's Reagent from weight of  $\text{Cu}_2\text{O}$ , thrown down, 0.250 gm. of the sugar being used in each test\*.**

d Glucose (Dextrose)			Invert Sugar†			Lactose (Cryst.)†			Maltose (Cryst.)		
Per Cent	$\text{Cu}_2\text{O}$ mg.†	Factor	Per Cent	$\text{Cu}_2\text{O}$ mg.†	Factor	Per Cent	$\text{Cu}_2\text{O}$ mg.†	Factor	Per Cent.	$\text{Cu}_2\text{O}$ mg.†	Factor
5	29.7	0.1736	5	28.0	0.1796	5	22.3	0.2778	5	17.6	0.3356
10	58.5	0.1758	10	56.0	0.1818	10	40.3	0.2762	10	32.5	0.3333
15	87.0	0.1786	15	83.5	0.1859	15	58.4	0.2778	15	47.5	0.3333
20	115.0	0.1812	20	110.4	0.1898	20	76.4	0.2778	20	52.4	0.3333
25	142.6	0.1838	25	137.8	0.1887	25	94.4	0.2778	25	77.4	0.3333
30	169.8	0.1852	30	164.3	0.1898	30	112.4	0.2793	30	92.4	0.3333
35	196.8	0.1887	35	190.6	0.1938	35	130.3	0.2778	35	107.4	0.3333
40	223.3	0.1916	40	216.4	0.1953	40	148.3	0.2909	40	122.4	0.3333
45	249.4	0.1938	45	242.0	0.1984	45	166.1	0.2793	45	137.4	0.3333
50	275.2	0.1969	50	267.2	0.2000	50	184.0	0.2793	50	152.4	0.3333
55	300.6	0.2000	55	292.2	0.2033	55	201.9	0.2809	55	167.4	0.3333
60	325.6	0.2033	60	316.8	0.2066	60	219.7	0.2793	60	182.4	0.3333
65	350.2	0.2058	65	341.0	0.2101	65	237.6	0.2825	65	197.4	0.3333
70	374.5	0.2092	70	364.8	0.2119	70	255.3	0.2825	70	212.4	0.3333
75	398.4	0.2137	75	388.4	0.2155	75	273.0	0.2825	75	227.4	0.3333
80	421.8	0.2155	80	411.6	0.2183	80	290.7	0.2825	80	242.4	0.3333
85	445.0	0.2193	85	434.5	0.2222	85	308.4	0.2841	85	257.4	0.3333
90	467.8	0.2332	90	457.0	0.2252	90	326.0	0.2841	90	272.4	0.3333
95	490.2	0.2273	95	479.2	0.2273	95	343.6	0.2825	95	287.4	0.3333
100	512.2	0.	100	501.2		100	361.3		100	302.5	

**803. Example:** Inverted sugar, representing 237.5 mg. of sucrose (= 250 mg. invert sugar) yields in test 485 mg. of  $\text{Cu}_2\text{O}$  (corrected figure). By the table nearest (lower) figure (479.2) corresponds with 95 percent invert sugar.  $485 - 479.2 = 5.8$  and  $5.8 \times 0.2273 = 1.32$ . Therefore percentage is  $95 + 1.32 = 96.32$ .

\*In case of sucrose, 0.2375 gm. are used equivalent to 0.250 gm. invert sugar.

†If copper is weighed in form of Cu. multiply these figures by 1.1259 to convert to  $\text{CuO}$ ; if weighed in form of  $\text{CuO}$ . Multiply by 1.1119.

‡Or sucrose, providing 0.2375 gm. of the sugar is used for the test, inverted according to (787).

‡For anhydrous lactose or maltose, multiply percent found by 0.95.

## Chapter X

### Digestive Ferments

#### DIASTASE

804. **The statement of the U. S. P. IX** is that diastase converts not less than 50 times its weight of potato starch into "sugars". It would be more correct to say that it "digests" under specified conditions 50 times its weight of anhydrous potato starch, using purposely a vague term. The test does not pretend to be strictly quantitative; it is a limitation test; and, since the product is described as subject to deterioration, it is obviously necessary to apply the test at intervals to any stock that may be on hand. The test may be so modified as to be a quasi-quantitative one. The procedure is identical with that described in (812, 813) except that the diastase solution to be tested is made to contain 0.2 gm. instead of 0.4 gm. in 100 mils. It must be borne in mind that this solution rapidly loses its activity and must therefore be made immediately before it is to be used.

#### PANCREATIN

805. **In pancreatin and similar preparations** we have to deal, not with definite chemical compounds, but with questions of enzyme activity which must be put to the test of actual experiment. As a rule such preparations are not only complex in composition, but exhibit activity in more than one line. Logically the standard for such a preparation must be selected with reference to the kind of activity which is of predominating therapeutic importance. The U. S. Pharmacopoeia requires in the case of pancreatin a minimum activity in two different directions; it must hydrolyze a certain quantity of starch under prescribed conditions, and it must "peptonize" a certain quantity of milk. It must be confessed that both tests are woefully lacking in definiteness.

**806. The milk test.** An obvious fallacy in this test is the assumption that "fresh milk" has an identity so well defined that it is suited to serve for a basis for a scientific standard. The present U. S. P. milk test appears at first sight to be an improvement on that given in U. S. P. VIII, the conditions being more exactly defined. There appears, however, to be an error in the quantity of acetic acid prescribed so that the test as it stands is worthless. It could serve at best only as a qualitative requirement, and this indeed is all that can be provided if the main requirement is to be quantitative with regard to the amylolytic activity of the product. Mr. Clarence P. Ramsay\* has proposed a new form of the proteolytic test which appears to be capable of yielding quite definite quantitative results. His proposed test depends on the fact that milk which has been completely peptonized by a tryptic enzyme is not coagulated by rennin. By a preliminary test an approximation is made to the datum sought, the exact figure being arrived at by a final series of more exact tests. For the tests, provide the following: (1) a solution (unfiltered) of 0.5 gm. of the pancreatin sample in distilled water sufficient to make 50 mls; (2) 900 mls of fresh cow's milk (not pasteurized) in which has been dissolved exactly 1.8 gm. of sodium bicarbonate; (3) 2 gm. of rennin (1:30,000 in 10 minutes, or equivalent) and exactly 1 mil of 6 percent acetic acid, added to 50 mls of distilled water.

**807. For the preliminary test,** measure into each of five cylindrical tubes of about 100 mls capacity, 50 mls of the milk, previously warmed to 40° C., measure into these tubes respectively 5, 6, 7, 8, and 9 mls of the pancreatin solution, corresponding respectively with 1:1000, 1:833, 1:714 and 1:555 as ratio of pancreatin to milk. Mix well and allow the pancreatin to act in each case exactly 1.5 minutes, then add to 5 mls of the digested milk in a test tube, 3 mls of the rennin solution and shake the mixture well. In the samples in which peptonization is complete, no

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\*Journ. Ind. & Eng. Chem., Nov. 1911, p. 822.

precipitate is produced, so that the strength of the pancreatin is fixed between certain limits. A new series of experiments must now be made to determine to any desired degree of exactness the tryptic activity of the sample. Note that the pancreatin solution must be used within half an hour after it is made.

808. The following table shows the quantity of the pancreatin solution corresponding with different strengths of the pancreatin.

TABLE

Mils of pancreatin solution	Strength of sample	Mils of pancreatin solution	Strength of sample
10.00	1:500	6.58	1:760
9.80	1:510	6.49	1:770
9.62	1:520	6.41	1:780
9.43	1:530	6.33	1:790
9.26	1:540	6.25	1:800
9.09	1:550	6.17	1:810
8.93	1:560	6.10	1:820
8.77	1:570	6.02	1:830
8.62	1:580	5.95	1:840
8.47	1:590	5.88	1:850
8.33	1:600	5.81	1:860
8.20	1:610	5.75	1:870
8.06	1:620	5.68	1:880
7.94	1:630	5.62	1:890
7.81	1:640	5.56	1:900
7.69	1:650	5.49	1:910
7.58	1:660	5.43	1:920
7.46	1:670	5.38	1:930
7.35	1:680	5.32	1:940
7.25	1:690	5.26	1:950
7.14	1:700	5.21	1:960
7.04	1:710	5.15	1:970
6.94	1:720	5.10	1:980
6.85	1:730	5.05	1:990
6.76	1:740	5.00	1:1000
6.67	1:750		

Rule: To find the figures in column II, multiply the reciprocal of the corresponding figure in column I by 5000. Thus if figure in column I were 4.00 (reciprocal 0.25) the strength of the sample would be 1:1250.

809. The Casein test has been improved by A.

R. Smith\*, so as to give reasonably quantitative results. A 4 percent solution of casein is prepared by dissolving 40 percent of casein (Hammarsten) in 900 mls of recently boiled and cooled distilled water containing 30 mls of normal sodium hydroxide. The solution is made exactly neutral to phenolphthalein and made up to 1 liter, chloroform being added as an antiseptic. A formaldehyde solution is also provided, prepared by adding to 50 mls of 40 percent formaldehyde, 1 mil of a solution of 0.5 gm. of phenolphthalein in 100 mls of 50 percent alcohol. Reaction of formaldehyde with amino products of tryptic digestion leaves free acids as a measure of the amount of digestive change.

810. One gm. of pancreatin is rubbed up in a mortar with a little chloroform water, washed into a 100 mil measuring flask and the volume made up to 100 mls with chloroform water. After standing one hour, 1 mil of the well mixed unfiltered solution is added to 25 mls of the casein solution, and the volume made up with water to 50 mls. The solutions are brought to a temperature of 55° C. before mixing, and the mixture is kept at this temperature 20 minutes. An aliquot part (20 mls) of the cooled solution is mixed with 20 mls of the formaldehyde solution, previously accurately neutralized with sodium hydroxide, and the mixture is titrated with fifth-normal sodium hydroxide. A second titration (blank) is made of a mixture identical with that submitted to digestion. The result of the blank is subtracted from that of the former titration, the difference indicating the digestive activity of the pancreatin, a standard sample showing about 1.28 mls.

811. **Pigs' fibrin is employed** in the method of the French Codex, and this method is given preference over those of other pharmacopoeias by R. Delaunay and O. Bailly†. The pigs' fibrin is prepared by washing, pressing out excess of water, drying at 40° C. and reducing to a powder. Place in a flask 60 mls of

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\*Year Book of Pharmacy, 1912, 525-33.

†Bull. Sci. Pharm., 1912, 19, 540.

distilled water, with 2.5 gm. of the fibrin; maintain a constant temperature of 50° C. 30 minutes, then add 0.2 gm. of the sample of pancreatin and digest at 50° C. six hours, shaking the mixture frequently until the fibrin is dissolved, after that shaking once every hour. Filter and to 10 mls of the filtrate add 1 mil of nitric acid, sp. gr. 1.394. No turbidity should be produced. In place of the dried fibrin, 10 gm. of moist pigs' fibrin may be employed, the quantity of water being reduced to 50 mls.

**812. Test for Amylolytic activity.** The "Assay" of U. S. P. IX is in fact only a minimum requirement. It serves its purpose, but it has no quantitative value. The following modification of it, suggested by Baker, gives it quantitative character and makes possible a definite standard, if such a standard is desirable. Potato starch is used for the test. This is to be repeatedly washed with water and then carefully dried at 50° C. In this condition it contains still perhaps 10 percent of moisture. The exact quantity of this is to be determined by drying a weighed sample (about 0.5 gm.) in an air bath at a temperature gradually raised to 120° C. and so maintained for 4 hours. Weigh a quantity of the starch equivalent to 10 gm. of anhydrous starch, and prepare from this exactly 500 gm. of starch paste (to be thoroughly gelatinized by 10 minutes boiling). Dissolve 0.4 gm. of pancreatin in 100 mls of water. Provide an iodine test solution prepared by diluting 2 mls of tenth-normal iodine solution with water to 50 mls, and diluting 5 mls of this to 150 mls.

**813.** Place in each of five test tubes 25 gm. of the starch solution. Heat these in a water bath to 40° C. and add to the test tubes respectively 4.0, 4.5, 5.0, 5.5 mls of the pancreatin solution; shake the mixture in each case and allow it to remain just 10 minutes, then withdraw 0.2 mls and add it to 5 mls of the iodine solution contained in a cylinder of white glass. In case transformation of the starch is complete (for the purposes of this test) no color will be produced, and it will be easy to calculate

the "digesting" power of the sample. If 5 mils of the pancreatin solution (0.4 percent) is the smallest quantity that yields a color free mixture, 0.02 gm. of the sample will "digest" 0.50 gm. or 25 times its weight of anhydrous starch, and that may be arbitrarily assumed as a standard for this product. A second series of tests, using 4.8, 4.9, 5.1 and 5.2 mils of the pancreatin solution, will enable us to fix quite precisely the amylolytic activity of the sample.

### PAPAIN (PAPAW JUICE)

814. **The testing of papaw juice** or the commercial product marketed as papain has been studied within recent years by H. T. Graber\*, J. R. Rippetoe†, M. M. Delauney and Bailly‡, H. M. Adams,§ Messrs. Heyl, Caryl and Staley||, and D. G. Pratt.†† A final assay method has not been reached. The following are as good as any yet proposed.

815. **Method of Thorburn**, approved by sub-committee on standardization and drug testing of A. Ph. A\*\*. The test excludes the possibility which vitiates many of the assay methods, viz: that pepsin may be present in the papain. The test is so arranged that pepsin if present is rendered inert by the preliminary digestion in an alkaline medium. Scrape to a pulp lean round steak, rejecting fat, gristle, etc. Place in a 200 mil digestion flask 10 gm. of the pulp, add a solution of papain 0.400 gm. and sodium bicarbonate 0.750 gm. in distilled water sufficient to make 100 mils, having warmed this solution to 50°—55° C.; digest 4 hours at 55° C., shaking the mixture once every 10 minutes, then cool rapidly, pour into a graduated cylinder and let stand half an hour. Not more than 10 mils of residue should remain. Warm the mixture once more to 55° C. and add strong

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\*Journ. Ind. & Eng. Chem., 1911, 921.

†Ibid. 1912, 517.

‡Bull. Sci. Pharm., 1913, 20, 141.

§Journ. Ind. & Eng. Chem., 1914, 669.

||Am. Journ. Pharm., 1914, 543.

††Philippine Journ. Sci., 1915, 10, 1.

\*\*Journ. Am. Pharm. Assoc., 1915, 224.

hydrochloric acid, sufficient to give to the fluid an acidity between 0.2 and 0.3 percent, and digest as before 4 hours at 55° C., shaking once in 10 minutes. The residue after standing at rest 30 minutes should measure less than 3 mils.

**816. Method of Heyl, Caryl and Staley.** Prepare an egg albumen solution from the separated whites of six eggs, by beating them, slightly diluting with twice the volume of a 1-percent solution of sodium chloride, and making up to a definite volume so that 15 mils shall contain 0.4 gm. of coagulable protein. Weigh into a 100 mil measuring flask 1 gm. of the papain, add some 1 percent sodium chloride solution, shake thoroughly and make up with more of the sodium chloride solution to 100 mils. After 30 minutes, measure into a dry 50 mil Erlenmeyer flask 1 mil of the papain solution, 15 mils of the standard albumen solution, and 9 mils of sodium chloride solution. Plunge the flask in a water bath at 80° C. and digest at that temperature exactly 15 minutes. Then add 1 mil of half normal acetic acid and heat 10 minutes in a boiling water bath. Collect the undigested albumen on a tared filter, wash free from chlorides with water, then wash once with 95 percent alcohol, and when this has drained, once with ether. Finally dry to constant weight at 100° — 105° and weigh. Run simultaneously a blank to determine the weight of coagulable albumen in the 15 mils of solution used, and so ascertain the exact amount of albumen digested (For the blank test not more than 5 mils of the albumen solution should be taken, with a proportionate amount of the salt solution and the acetic acid.)

## PEPSIN

**817. Any standard for pepsin** must be based on experiment to ascertain the actual digestive activity, under prescribed conditions, of the sample. Anything like scientific exactness cannot be hoped for in such tests, since there are so many factors entering into the case that it is hardly possible to exclude in the prescribed conditions every disturbing element. The method most commonly used is to ascertain approxi-



mately how much of the pepsin is required to dissolve 10 gm. of hard boiled white of egg, in a specified quantity, of water acidulated with hydrochloric acid. It has been found that the age of the egg is a matter of importance. An egg eight days old is more digestible (by pepsin) than one which is less than five or more than twelve days old. The proportion of acid in the solution is not less important. The best results are reached when that proportion is 0.3 percent. Even a moderate increase in the acidity has a decided effect in inhibiting the action of the pepsin.

818. **The assay of the U. S. P. IX** prescribes the conditions which have been found most favorable. The egg, which must be not less than five nor more than twelve days old, and must have been kept in a cool place, is immersed in boiling water fifteen minutes. When cool enough to handle, the separated white of the egg is forced through a No. 40 sieve, and 10 gm. of it ( in the moist condition) are weighed for the test, which is made in a bottle of 100 mls capacity (shape not specified). The white of egg is brought into uniform suspension in 35 mls of twelfth-normal hydrochloric acid, and 0.0333 gm. of pepsin dissolved in 5 mls of acid of the same strength is added. The temperature of the mixture is to be 52° C., and this temperature is maintained by means of a water bath during 2½ hours, the bottle being taken out and **inverted once** at intervals of ten minutes. The fluid with any particles which remain undigested of the egg albumen is transferred to a conical graduated measure and at the end of half an hour the volume of the deposit of undissolved albumen is read off. If the pepsin is of official strength (1 : 3000) this volume will not exceed 1 mil. If the exact digestive activity of the sample is to be ascertained, a number of tests must be carried out simultaneously, employing e. g. 0.02, 0.025, 0.03, 0.035, 0.04 and 0.05 gm. of pepsin, with a volume in each case of 40 mls of acidulated water. The strength of the pepsin is thus fixed between certain limits, and a second and if necessary a third series of tests will define these limits with any desired degree of exactness.

819. **Regarding the test**, it is to be remarked (1) that egg albumen in the condition prescribed must vary considerably in its content of water, (2) that mere "dissolving" of the coagulated albumen is not an indication of the actual result of the peptonizing process. (3) a rough measurement of the residue as prescribed lacks the exactness expected in a quantitative test.

820. **An alternative process** has been suggested\* aiming to give greater precision to the test. The egg albumen is "scaled" by drying on plates of glass at 40° C., and previous to use is powdered, and its content of water is determined by drying a portion of it at 100° C. Triturate about 1.2 gm. (accurately weighed) of the powder with 2 successive portions (10 mls) of warm water, transfer to a flask and heat 15 minutes in a boiling water bath to coagulate the albumen. Cool to 40° C. and add 0.25 gm. of pepsin dissolved in 25 mls of tenth-normal hydrochloric acid. Shake the mixture vigorously and immerse in a water bath at a regulated temperature of 40° C., 4 hours, shaking every half hour. Then immerse the flask in boiling water half an hour to prevent further action of the pepsin. Cool to 15° and make up to 100 mls with water, shake and transfer to a test tube 10 mls of the solution and add 13 gm. of zinc sulphate, and 0.23 mls of diluted sulphuric acid (114). Heat the mixture to boiling and filter through a dry filter. Transfer 5 mls of the solution to a Nesslerizing tube, add 15 mls of water, 1 mil of a 0.5 percent solution of crystallized copper sulphate with solution of sodium hydroxide (30 percent) sufficient to make 80 mls and filter through glass wool. For color comparison add to 75 mls of distilled water, recently boiled and cooled, a solution of potassium permanganate (0.04 gm. to 1 liter) to match the color of the prepared peptone solution.

821. **The details of the test** require modification particularly as regards the quantity of acid used and the temperature to be maintained during digestion,

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\*W. B. Cowie and W. Dickson, in *Pharm. Journ.*, 1906, 221-3

and a numerical value is lacking for interpreting the result of the test, but if the principle of the assay is sound these details can be worked out.

**822. Fibrin seems better suited** for the purposes of such a test than egg albumen. This is employed (pig fibrin) in the assay of the French Codex which is made in the following manner: Put into a wide mouthed flask 2.5 gm. of dried fibrin with 58.5 gm. of distilled water and 1.5 gm. of diluted hydrochloric acid (10 percent), heat the mixture to 50° C., add 0.1 gm. pepsin, and digest at a constant temperature of 50° C. six hours, shaking frequently until the fibrin is dissolved, afterward at intervals of about one hour. Ten mls of the cooled and filtered liquid are not rendered turbid by 1 mil of nitric acid (sp. gr. 1.394). The digestive power of a sample of pepsin not of official standard is to be ascertained by a series of tests in which varying quantities of the pepsin are used.

**823. Edestin, a crystallizable nitrogenous compound** found in hemp seed, on account of its definiteness of chemical composition, is believed to be preferable even to fibrin\*. The edestin must be in crystallized form. A filtered solution of 0.5 gm. of this with 0.25 gm. of hydrochloric acid in 100 mls of distilled water is used for the test. Twenty mls of this solution are transferred to a test tube which is immersed during 10 minutes in a water bath, kept at 50° C.; 0.02 gm. of pepsin is then added and the temperature maintained at 50° C. exactly 15 minutes. The tube is then plunged in water at 17° C. and treated at once with 1.5 mls of nitric acid, sp. gr. 1.394, which causes no turbidity if the pepsin is of official (Codex) strength. (In place of nitric acid, pure sodium chloride, dissolved in the solution to near the point of saturation, may be used. This will produce no precipitate if digestion of the edestin is complete.)

**824. The Rose method**, in which pea globulin is used in place of edestin has been studied by M. H. Givens†, and details are given by him of its application

\*Delauny and Bailly, Bull. Sci. Pharm., 1915; Year-book of Brit. Pharm. Conf., 1915, 31.

†Am. Journ. Pharm., 1915, 541.

in testing the gastric secretion. The pea globulin is prepared according to Rose in the following manner: The finely ground peas, freed as much as possible from the outer covering, are repeatedly extracted with 10 percent solution of sodium chloride, the extracts combined, strained through fine bolting cloth and allowed to stand over night to deposit insoluble matter. The clear liquid is decanted or siphoned off and saturated with ammonium sulphate. The precipitate is suspended in a little water and dialyzed 3 days to remove the salts, and the globulin precipitate is freed from albumins by washing repeatedly with water. The precipitate is finally dried at 45° C.

825. Another vegetable educt, ricin, a toxalbumin from castor oil seeds, has been used in a similar manner. For details see *Journal A. O. A. C.*, November 1916, Part II, p. 363.

826. An important consideration in all pepsin tests is the inhibitory influence on digestion of various substances which may be present. A comprehensive study of this important subject has been made by C. F. Ramsay\*. Most mineral salts were found to have a decided influence—greater in the case of salts of calcium and magnesium than in those of the alkalis. Even sodium chloride in the quantity of 0.06 gm. reduced the activity of pepsin by the U. S. P. test 40 percent, while 0.0025 gm. of magnesium sulphate caused a 17 percent reduction. Small quantities of sugar, glycerin and even of alcohol had no effect, but 0.1 mil of chloroform almost completely prevented digestion. Neither spice nor caffeine caused inhibition, but tannic acid and salts of quinine (notably the sulphate), of morphine and of nicotine had a pronounced injurious action, and in a still greater degree, saccharin.

827. In the examination of medicinal tablets in which pepsin is combined with calcium carbonate, a negative result is to be expected unless separation of these constituents is effected by preliminary treatment.

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\**Journ. Am. Pharm. Assoc.*, 1916, 5-30.

# ADDENDUM

## Alkalimetry up to Date

328. Within the very recent past, alkalimetry has been placed as never before on a thoroughly scientific basis. New indicators have been discovered, superior in every way to any heretofore known, notably those having for their basis phenolsulphophthalein\*.

329. The object sought in acidimetric titrations is the accurate determination of H-ion concentration, each indicator having its own views with regard to the "neutrality" point as shown by change of color. As a result of two years of persevering endeavor along scientific lines, H. A. Lubs in collaboration with Dr. W. M. Clark† of the Bureau of Animal Industry, have developed a series of new and brilliant indicators covering a wide range of H-ion concentration. Of especial interest among these must be mentioned: thymosulphophthalein, which has the unique peculiarity of showing two distinct sharp color changes in passing from acidity to alkalinity, the original red changing before the acid is quite neutralized to yellow, further addition of alkali changing the color finally to blue.

330. Accurate determination of H-ion concentration is now made without use of any indicator, simply by observing the change in electrical conductivity of the solution at the moment of neutralization. The apparatus required is, however, so elaborate and costly that the method cannot well be applied in pharmaceutical practice.

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\*See paper by H. A. Lubs in *Jn. Ind. & Eng. Chem.* 1920, 273; also studies of certain indicators by E. C. White and S. F. Acres, *Jn. Am. Chem. Soc.* 1916, 648; 1917, 1092 and 1919, 1190 (summary of conclusions, *ibid.* pp. 1211-2.) Additional references are (on work of Sorenson and his associates) *Compt. rend. trav. Lab. Carlsberg*, 1902, 1; 1909, 1; *Biochem. Z.* 1909, 131, 209; 1913, 307. *Erzebn, Physiol.* 1912, 393; (Researches of Rowntree) *Archiv; Intern. Med.* 1915, 38.

†*Jn. Bact.* 1917, 1, 109, 191.

831. Of great practical importance, however, are some of the improvements recently made in the method of determining accurately the end point in an alkalimetric titration, particularly when the solution under examination is colored. The work of Dr. Alfred Tingle is especially noteworthy. He recommends the use of the pocket spectroscope for this purpose. With many indicators the color change involves easily observed changes in the absorption bands seen in the spectrum. Such changes occur in the case of some of the indicators employed in alkaloidal titrations, notably cochineal, methyl orange and methyl red. An acid solution, for example, colored with methyl red shows a strong absorption band in the green region, which disappears sharply the moment the solution is made just neutral. The fluid under examination is placed in a container having parallel sides so that the distance traversed by the light ray in passing through the solution shall be always approximately the same. The methyl red solution may conveniently be of a strength of 1:2000. Of this 3 mils will suffice for 100 mils of solution, a quantity larger than is required when the titration is made in the usual manner. The diameter of the cell containing the fluid should be 45 to 50 mm.

832. The instrument recommended is the Beck-Thorp patent reading pocket diffraction spectroscope. For details of the method and its applications, the original papers should be consulted in the *Journal of the American Chemical Society* for June 1918, pp. 872-9, and in the *Journal of Industrial and Engineering Chemistry* for March 1920, pp. 274-6.

# Molecular Formulas and Weights of Principal Items considered in foregoing pages:

Acetaldehyde, $\text{CH}_3\text{CHO}$ .....	44.03
Acetanilid, $\text{C}_8\text{H}_9\text{ON}$ .....	135.08
Acetic Anhydride $(\text{CH}_3\text{CO})_2\text{O}$ .....	102.05
Acetone $\text{C}_3\text{H}_6\text{O}$ .....	58.05
Acetphenetidin, $\text{C}_{10}\text{H}_{13}\text{O}_2\text{N}$ .....	179.11
Acid Acetic, $\text{HC}_2\text{H}_3\text{O}_2$ .....	60.03
Acid Acetylsalicylic, $\text{HC}_7\text{H}_4\text{O}_2\text{C}_2\text{H}_3\text{O}_2$ .....	180.06
Acid Benzoic, $\text{HC}_7\text{H}_5\text{O}_3$ .....	122.05
Acid Cinnamic, $\text{HC}_9\text{H}_7\text{O}_2$ .....	148.06
Acid Citric, $\text{H}_3\text{C}_6\text{H}_5\text{O}_7 + \text{H}_2\text{O}$ .....	210.08
Acid Citric, Anhydrous, $\text{H}_3\text{C}_6\text{H}_5\text{O}_7$ .....	192.06
Acid Formic, $\text{HCHO}_2$ .....	46.02
Acid Gallic, $\text{HC}_7\text{H}_5\text{O}_5 + \text{H}_2\text{O}$ .....	188.06
Acid Glycerophosphoric, $\text{H}_2\text{C}_3\text{H}_5(\text{OH}_2)\text{PO}_4$ ...	172.11
Acid Lactic, $\text{HC}_3\text{H}_5\text{O}_3$ .....	90.05
Acid Oleic, $\text{HC}_{18}\text{H}_{33}\text{O}_2$ .....	282.27
Acid Picric (Trinitrophenol), $\text{C}_6\text{H}_3\text{O}_7\text{N}_3$ .....	229.05
Acid Picrolonic, $\text{C}_{10}\text{H}_8\text{O}_5\text{N}_4$ .....	264.10
Acid Salicylic, $\text{HC}_7\text{H}_5\text{O}_3$ .....	138.05
Acid Tannic (variable), $\text{HC}_{14}\text{H}_9\text{O}_9$ .....	[322.08]
Acid Tartaric, $\text{H}_2\text{C}_4\text{H}_4\text{O}_6$ .....	150.05
Aconitine, $\text{C}_{34}\text{H}_{47}\text{O}_{11}\text{N}$ .....	645.39
Alcohol, Amyl, $\text{C}_5\text{H}_{11}\text{OH}$ .....	88.10
Alcohol, Ethyl, $\text{C}_2\text{H}_5\text{OH}$ .....	46.05
Alcohol, Methyl, $\text{CH}_3\text{OH}$ .....	32.03
Aldehyde, Acetic, $\text{CH}_3\text{CHO}$ .....	44.03
Aldehyde, Benzoic, $\text{C}_6\text{H}_5\text{CHO}$ .....	106.05
Aldehyde, Cinnamic, $\text{C}_8\text{H}_7\text{CHO}$ .....	132.06
Aldehyde, Formic, $\text{HCHO}$ .....	30.02
Allyl Isothiocyanate, $\text{C}_3\text{H}_5\text{SCN}$ .....	99.12
Amyl Nitrite, $\text{C}_5\text{H}_{11}\text{NO}_2$ .....	117.10
Anethol, $\text{C}_{10}\text{H}_{12}\text{O}$ .....	148.10
Aniline, $\text{C}_6\text{H}_5\text{NH}_2$ .....	93.07
Aniline Sulphate, $(\text{C}_6\text{H}_5\text{NH}_2)_2\text{H}_2\text{SO}_4$ .....	284.22

Antipyrine, $C_{11}H_{12}ON_2$ .....	188.12
Aspidospermine, $C_{22}H_{30}O_2N_2$ .....	354.26
Atropine, $C_{17}H_{22}O_3N$ .....	289.19
Atropine Sulphate, $(C_{17}H_{22}O_3N)_2H_2SO_4 + H_2O$ .....	694.49
Benzaldehyde, $C_6H_5CHO$ .....	106.05
Benzene, $C_6H_6$ .....	78.05
Betanaphthol, $C_{10}H_8O$ .....	144.06
Borneol, $C_{10}H_{17}OH$ .....	154.14
Bornyl Acetate, $C_{10}H_{17}C_2H_3O_2$ .....	196.16
Bromoform, $CHBr_3$ .....	252.77
Brucine, Anhydrous, $C_{23}H_{26}O_4N_2$ .....	394.23
Caffeine, Anhydrous, $C_8H_{10}O_2N_4$ .....	194.12
Camphor, $C_{10}H_{16}O$ .....	152.13
Camphor Monobromated, $C_{10}H_{15}OBr$ .....	231.04
Carvone, $C_{10}H_{14}O$ .....	150.11
Cephaeline, $C_{14}H_{19}O_2N$ .....	233.16
Chloral, Hydrated, $C_2HCl_3O + H_2O$ .....	165.40
Chloroform, $CHCl_3$ .....	119.39
Cinchonidine, $C_{19}H_{22}ON_2$ .....	294.20
Cinchonine, $C_{19}H_{22}ON_2$ .....	294.20
Cineol (Eucalyptol), $C_{10}H_{18}O$ .....	154.14
Cinnamic Aldehyde, $C_9H_8O$ .....	132.06
Citral, $C_{10}H_{16}O$ .....	152.13
Cocaine, $C_{17}H_{21}O_4N$ .....	303.18
Codeine, Anhydrous, $C_{18}H_{21}O_3N$ .....	299.18
Colchicine, $C_{22}H_{25}O_6N$ .....	399.21
Coniine, $C_8H_{17}N$ .....	127.15
Cotarnine, $C_{12}H_{15}O_4N$ .....	237.13
Coumarin, $C_9H_6O_2$ .....	146.05
Cresol $C_6H_4(CH_3)OH$ .....	108.06
Diacetyl-morphine, $C_{21}H_{23}O_5N$ .....	369.19
Diethyl-barbituric Acid, $C_8H_{12}O_3N_2$ .....	184.12
Dithymol-Diiodide, $(C_{10}H_{12}O)_2I_2$ .....	550.03
Emetine, $C_{15}H_{22}O_2N$ .....	248.19
Epinephrine, Hydrated, $C_9H_{13}O_3N + 1\frac{1}{2}H_2O$ .....	192.12
Ether (Ethyl Oxide), $(C_2H_5)_2O$ .....	74.08
Ether, Acetic (Ethyl Acetate), $C_2H_5C_2H_3O_2$ ..	88.06
Ethyl Nitrite, $C_2H_5NO_2$ .....	75.05
Eucaïne (Beta), $C_{15}H_{21}O_2NHCl + H_2O$ .....	301.66
Eucalyptol (Cineol), $C_{10}H_{18}O$ .....	154.14
Eugenol, $C_{10}H_{12}O_2$ .....	164.10
Formaldehyde, $HCHO$ .....	30.02



Fuchsin, $C_{20}H_{19}N_3HCl$ .....	337.74
Glucose, $C_6H_{12}O_6$ .....	180.10
Glycerol, $C_3H_5(OH)_3$ .....	92.06
Glyceryl trinitrate, $C_3H_5(NO_2)_3$ .....	227.07
Guaiacol, $C_7H_8O_2$ .....	124.06
Hexamethylenamine, $C_6H_{12}N_4$ .....	140.14
Hydrastine, $C_{21}H_{21}O_6N$ .....	283.18
Hydrastinine, $C_{11}H_{11}O_2N$ .....	189.10
Hyoscyne, $C_{17}H_{21}O_4N$ .....	303.18
Hyoscyamine, $C_{17}H_{23}O_3N$ .....	289.19
Iodoform, $CHI_3$ .....	393.77
Lactose, $C_{12}H_{22}O_{11} + H_2O$ .....	360.19
Lecithin (variable) $C_{42}H_{84}O_9NP$ .....	[777.72]
Limonene, $C_{10}H_{16}$ .....	136.13
Linallyl Acetate, $C_{10}H_{17}C_2H_3O_2$ .....	196.16
Lobeline, $C_{18}H_{23}O_2N$ .....	285.19
Menthol, $C_{10}H_{19}OH$ .....	156.16
Menthyl Acetate, $C_{10}H_{19}C_2H_3O_2$ .....	198.18
Methyl Salicylate, $CH_3C_7H_5O_3$ .....	152.06
Morphine, Anhydrous, $C_{17}H_{19}O_3N$ .....	285.16
Morphine, Cryst $C_{17}H_{19}O_3N + H_2O$ .....	303.18
Naphthol, $C_{10}H_8O$ .....	144.06
Nitroglycerin, $C_3H_5(NO_2)_3$ .....	227.07
Nitron, $C_{20}H_{16}N_4$ .....	312.27
Ouabain, $C_{30}H_{46}O_{12}$ .....	760.51
Phenol, $C_6H_5OH$ .....	94.05
Phenolphthalein, $C_{20}H_{14}O_4$ .....	318.11
Phenylhydrazine, $C_6H_5NHNH_2$ .....	108.08
“ Hydrochloride, $C_6H_5NHNH_2HCl$ ...	144.55
Phenyl Salicylate, $C_6H_5C_7H_5O_2$ .....	214.08
Physostigmine, $C_{15}H_{21}O_2N_3$ .....	275.20
Picrotoxin, $C_{30}H_{34}O_{13}$ .....	602.27
Pilocarpine, $C_{11}H_{16}O_2N_2$ .....	208.15
Piperine, $C_{17}H_{19}O_3N$ .....	285.16
Pyrogallol, $C_6H_3(OH)_3$ .....	126.05
Quinidine, $C_{20}H_{24}O_2N_2$ .....	324.21
Quinine, Anhydrous, $C_{20}H_{24}O_2N_2$ .....	324.21
Resorcinol, $C_6H_4(OH)_2$ .....	110.05
Saccharin, $C_7H_5O_3NS$ .....	183.12
Safrol, $C_{10}H_{10}O_2$ .....	162.08
Salicin, $C_{13}H_{18}O_7$ .....	286.14
Salol, $C_6H_5C_7H_5O_2$ .....	214.08

Santalol, $C_{15}H_{26}O$ . . . . .	222.21
Santonin, $C_{15}H_{18}O_3$ . . . . .	246.14
Scopolamine, $C_{17}H_{21}O_4N$ . . . . .	303.18
Starch, Anhydrous, $C_6H_{10}O_5$ . . . . .	162.08
Strophanthidin, $C_{27}H_{38}O_7 + 2H_2O$ . . . . .	618.34
Strophanthin, $C_{40}H_{66}O_{19} + 3H_2O$ . . . . .	904.57
Strychnine, $C_{21}H_{22}O_2N_2$ . . . . .	334.20
Sucrose, $C_{12}H_{22}O_{11}$ . . . . .	342.18
Sugar, Cane, $C_{12}H_{22}O_{11}$ . . . . .	342.18
Sugar, Grape, $C_6H_{12}O_6$ . . . . .	180.10
Sugar, Malt, $C_{12}H_{22}O_{11} + H_2O$ . . . . .	360.19
Sugar, Milk, $C_{12}H_{22}O_{11} + H_2O$ . . . . .	360.19
Sulphomethylmethane, $C_8H_{18}O_4S_2$ . . . . .	242.28
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Trinitrophenol, $C_6H_3O_7N_3$ . . . . .	229.05
Vanillin, $C_8H_8O_3$ . . . . .	152.06

## Apparatus Required for Chemical Assay of Organic Drugs and Galenicals

A. **Items indispensable** for equipment of ordinary pharmacists' laboratory. Many of these are to be found of course in any up to-date drug store. The few marked with an asterisk may be considered not absolutely necessary.

A good laboratory balance sensitive to 1 mg. with load of 100 gm.

Set of accurate metric weights from 20 gm. down\*

Metric measuring flasks, 1,000, 500, 250, 200, 100, 50, 25 and 10 mls.

Metric graduates (cylindrical), 100, 50, 25 and 10 mls.

Metric measuring pipettes, 100, 50, 25, 12.5, 10, 5, 2 and 1 mil.

Metric graduated pipettes, 20, 10, 5 and 1 mil, graduated to tenths or twentieths.

Burette (preferably 2), with stand, 50 or 25 mls, graduated to tenths.

Separating funnels (separators), at least 5, preferably 100 mil.

Beakers, 500, 250, 100, 50 and 25 mil.

Erlenmeyer flasks, 250, 150, 100, 50 and 25 mil.

Distilling flasks, 500 and 250 mil, with receivers.

Condensers, direct and reflux.

Retort stand.

Support (tripod) for beaker, flasks, etc., with wire gauze and asbestos board.

Water bath.

Sand bath or hot plate.

Evaporating dishes (porcelain), flat, 6 to 15 cm. in diameter.

Glass tubing, assorted, 1 cm. down.

Rubber tubing for connections.

Test tubes and rack.

Filtering funnels, 60° angle, 4 to 20 cm. diameter.

Filters, 25 to 6 cm. diameter.

Washing bottle.

Crucibles, porcelain for fusions, 000 and 0.

Specific gravity bottle, 25 gm.

Thermometers, centigrade.

Bunsen burner, preferably of Meker or Scimatco type. (In absence of gas, alcohol or vaporized oil burner.)

**B. Additional items** for fully equipped laboratory. This list may be extended indefinitely. Only the items of prime importance are included.

A good analytical balance (college model or better).

Apparatus for continuous extraction, soxhlet or its equivalent.

Apparatus for nitrogen determination (Kjeldahl).

Artificial daylight burner.

Apparatus for continuous extraction of liquids ("perforator").

Acetylation flask.

Babcock milk testing apparatus.

Cassia flask.

Desiccator, vacuum.

Distilling apparatus, vacuum.

Drying oven (regulated temperature).

Filter pump.

Centrifuge.

Colorimeter.

Crucible, Gooch.

Hydrometers, including standard set for alcohol determinations.

Melting point apparatus.

Nitrometer.

Platinum (or alloy) crucible and evaporating dish.

Pressure bottle.

Polariscope.

Refractometer.

Shaking apparatus.

Viscometer.

Consult catalogues of leading dealers in making selections for purchase.

## REAGENTS

It is needless to emphasize the importance of a high degree of purity in all chemicals purchased for use as reagents. Such chemicals are now readily procurable (analyzed reagents) being supplied by dealers in chemical apparatus as well as by the large wholesale drug houses. The U. S. Pharmacopoeia gives a full list of the reagents, test solutions and volumetric solutions required for pharmacopoeial tests, with full instructions for preparing such solutions.

The following list includes the more important reagents required in the tests described in this book which are likely to be lacking in the stock of the pharmacist:

Acetic Anhydride  
Acetone  
Aniline Sulphate  
Azolitmin  
Benzene  
Dinitrophenolphthalein  
Fluorescein  
Fuchsin  
Glucose, Dry  
Hematoxylin  
Iodeosin  
Lacmoid  
Methyl Red  
Ouabain  
Petroleum Ether, Redistilled\*  
Phenylhydrazine  
Phenylhydrazine hydrochloride  
P-nitrophenol  
Sawdust, Purified  
Sodium Tungstate  
Tetrachlor-tetrabrom-phenolphthalein  
Thymosulphophthalein.  
Uranin

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\*Boiling below 70° C.

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